Experimental models of the respiratory distress syndrome
Lavage and oleic acid

Experimentele modellen van acute respiratoire insufficiëntie
Lavage en oliezuur

PROEFSCHRIFT

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CHAPTER I

GENERAL INTRODUCTION

The respiratory distress syndrome is manifested by an acute onset of tachypnea, hypoxemia and decrease of respiratory system compliance without a history of chronic lung disease or heart failure (2). Two main syndromes of respiratory distress are known: adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS). Both will be described in this chapter.

Adult respiratory distress syndrome

Definition and clinical picture

In 1967 Ashbaugh et al. (2) described 12 adult patients with a sudden onset of dyspnea, hypoxemia, reduced respiratory system compliance and diffuse alveolar infiltrates on the chest roentgenogram, that resembled pulmonary edema without evidence of prior lung disease or congestive heart failure. Shortly afterwards, Petty et al. (83) called this entity of disorders adult respiratory distress syndrome (ARDS). Other names, which have been used for this syndrome, are shock lung, protein-rich lung edema, post-traumatic pulmonary insufficiency and DaNang lung. Usually there is a latent period of 12-48 h. for the development of ARDS after the initial insult (3). Besides the symptoms mentioned by Ashbaugh et al. (2), functional residual capacity is decreased and venous admixture and physiological dead space are increased (46).

Petty (84) proposed criteria for diagnosing ARDS. An important physiological criterion is hypoxemia with an oxygen tension in arterial blood (P_{aO2}) of less than 50 mmHg with an inspiratory oxygen fraction (F_{IO2}) greater than 0.6. Murray et al. (72) introduced a numerical score to determine the severity of lung injury in ARDS patients. The components of this score system are the chest roentgenogram score, hypoxemia score, positive end-expiratory pressure score and respiratory system compliance score. In Table 1.1 this lung injury score is presented in detail. Nowadays, the criteria by Murray et al. (72) are most frequently used in patient studies.

ARDS has been reported to complicate a variety of intra- and extra-

Table 1.1. Lung injury score.

1. Chest roentgenogram score			
No alveolar consolidation			0
Alveolar consolidation confined to 1 quadrant			1
Alveolar consolidation confined to 2 quadrants			2
Alveolar consolidation confined to 3 quadrants			3
Alveolar consolidation in all 4 quadrants			4
2. Hypoxemia score			
P_{aO2}/ F_{IO2}	$\geq$ 300	mmHg	0
P_{aO2}/ F_{IO2}	225-299	mmHg	1
P_{aO2}/ F_{IO2}	175-224	mmHg	2
P_{aO2}/ F_{IO2}	100-174	mmHg	3
P_{aO2}/ F_{IO2}	$\leq$ 99	mmHg	4
3. PEEP score			
PEEP	$\leq$ 5	cmH_2O	0
PEEP	6-8	cmH_2O	1
PEEP	9-11	cmH_2O	2
PEEP	12-14	cmH_2O	3
PEEP	$\geq$ 15	cmH_2O	4
4. Respiratory system compliance score			
Compliance	$\geq$ 1.14	$ml.cmH_2O^{-1}.kg^{-1}$	0
Compliance	0.86-1.13	$ml.cmH_2O^{-1}.kg^{-1}$	1
Compliance	0.57-0.85	$ml.cmH_2O^{-1}.kg^{-1}$	2
Compliance	0.29-0.56	$ml.cmH_2O^{-1}.kg^{-1}$	3
Compliance	$\leq$ 0.28	$ml.cmH_2O^{-1}.kg^{-1}$	4

Modified from Murray et al. (72). Respiratory system compliance was recalculated to the value per kg body weight assuming an average weight of 70 kg of the patients.
By dividing the aggregate sum by the number of components the final score is obtained.

Score

0	: No lung injury
0.1-2.5	: Mild-to-moderate lung injury
>2.5	: Severe lung injury (ARDS)

pulmonary injuries as sepsis, pneumonia, near-drowning, aspiration of gastric contents, major trauma, fat embolism, thromboembolism, inhalation of toxic substances, drug overdose, multiple transfusions and acute pancreatitis (30,78,133). From these causes sepsis and gastric aspiration are most frequently followed by ARDS (30,78). Of all patients with a clinical predisposition 7-34 % actually develop the syndrome (30,66,78). One of the reasons for this range in percentage could be differences between inclusion and exclusion criteria of the different studies. Besides ARDS, the occurrence of multiple organ failure (MOF) is a frequent observation (77). It is not yet completely clear whether ARDS is mainly a lung disorder that causes non-pulmonary organ failure or a generalized disease that firstly becomes manifest in the lungs. Currently, ARDS is seen as a local expression of a generalized disease (60).

In the U.S.A. about 150,000 cases of ARDS per year occur (30,78). Transforming this number to the population of the Netherlands results in an estimation of about 10,000 ARDS patients annually. Despite improvements in supportive intensive care for ARDS patients, recent studies continue to report mortality rates between 40 and 75 % (30,63,78). Most patients who do not survive die within 14 days of onset of the syndrome (30). However, mortality rate may have been obscured by different entry criteria used for ARDS. As care has improved, the criteria may also have selected a smaller percentage of all patients with ARDS-like lung injury.

Stages of ARDS

Morphologically the evolution of ARDS can be divided into three phases (52,118,119).

1. Injury phase. This phase, which develops in the first week, is characterized by an alveolar, capillary and epithelial injury and increased permeability. The lungs of patients who die in this phase are heavy, airless and diffusely red-blue. Histologically interstitial edema, intra-alveolar hemorrhage, hyaline membranes and condensed fibrin are found.

2. Reparative and proliferative phase. This phase extends from the second to the third week and is characterized by interstitial inflammation, pneumocyte II cell regeneration and organisation. The lungs are more consolidated than in phase 1 and patchy red-brown and yellow-grey areas appear. Microscopically, there is proliferation of epithelial cells and organising granulation tissue.

3. Fibrotic phase. Interstitial collagen formation and architectural obliteration are phenomena that are found after the third week. Macroscopically, the lungs have about the same appearance as in phase 2, but fine cysts with air spaces of up to 1 mm appear. Histologically fibrous tissue, and distorsion and obliteration of alveolar and bronchiolar spaces exist.

Pathophysiology of ARDS

In spite of much research on the pathogenesis of ARDS, the precise mechanism is not known. ARDS can be triggered by many factors, which probably have a final common pathway.

Impairment of the alveolar-capillary membrane, resulting in an increased microvascular permeability, is central in the pathophysiology of ARDS. An important event seems to be the locally triggered production by pulmonary cells of substances, that are chemotactic for polymorphonuclear granulocytes (PMN) (64,114,115,127). Products of alveolar macrophages with chemotactic activity are the interleukines 1 and 8 (IL-1 and IL-8), tumor necrosis factor (TNF), platelet-activating factor (PAF) and platelet-derived growth factor (PDGF). Epithelial cells can form PDGF, IL-8 and lipoxygenase products and endothelial cells can form IL-1 and IL-8 (97,106,109). In sepsis induced ARDS endotoxin is regarded as an important trigger of the cellular reaction (92). Endotoxin can activate complement C5a, which stimulates PMN's and macrophages. Endotoxin can also directly stimulate macrophages to produce TNF, 5-hydroxyeicosatetraenoic acid (5-HETE), leukotriene B4 (LTB4) and thromboxane A2 (TxA2) (96). PAF might serve as a priming factor in endotoxin-induced TNF synthesis (89). TNF promotes the adherence of PMN's to endothelial cells and probably mediates thrombosis in the lung and diffuse intravascular coagulation (75,107). PMN's probably cause injury to the alveolar-capillary membrane by oxygen toxic products and toxic substances such as leukotrienes and proteolytic enzymes (19,65,93,94,102,112,114,115). The coagulation cascade triggers platelet activation with release of mediators (9). Blood coagulation factors have been suggested as mediators of lung injury (126).

A combination of edema and atelectasis causes ventilation-perfusion abnormalities. Atelectasis can be caused by abnormal surfactant, which has been shown to be functionally and biochemically abnormal mainly in the early phase of ARDS (42). Alterations in surfactant may be due to abnormalities in pneumocyte II cells or due to the presence of inhibitors in the alveolar lining fluid such as albumin (44), fibrin monomer (100) and hemoglobin (45). Oxygen radicals can cause injury to both the pneumocytes II and surfactant, also resulting in an abnormal surfactant function.

Pulmonary vessels in ARDS

Patients with ARDS usually have pulmonary hypertension. In the early stage pulmonary hypertension can be reversed by vasodilators, indicating that active vasoconstriction is an important factor (132). Active vasoconstriction in ARDS can be caused by hypoxia and by metabolites from thrombocytes and granulocytes. Endothelial cell swelling, thromboembolism and interstitial edema

cause obstruction of the lumen of the vessels (118).

After a long period of respiratory distress and mechanical ventilation, the arteries show tortuosities and all parts of the arterial wall are compromised. Endothelial cell swelling, thickening of the basement membrane, fibrocellular intimal proliferation and medial hypertrophy appear (118). With increasing duration of ARDS, an increase in arterial medial thickness as percentage of total vessel diameter occurs (104,118). This has been attributed to medial hypertrophy of arteries and muscularisation of the arterioles. All factors mentioned above can cause pulmonary hypertension and right ventricular failure (131). In later stages, pulmonary infarcts as well as dilation of capillaries can be found (119). Endothelial cell injury occurs in all stages regardless of the cause of the disease.

Focally, the pulmonary veins show intimal proliferation, which may contribute to an elevation of microvascular pressure. Obstruction of lymphatics secondary to edema further impedes removal of interstitial fluid (118).

Pulmonary sequelae of ARDS

Survivors of ARDS can be asymptomatic with a normal lung function, but can also have moderate to severe abnormalities in lung function. These abnormalities consist of disorders in diffusion, airway obstruction, restriction and increased pulmonary tissue stiffness (22,23,24,35,81,129,130). Supplemental oxygen, positive airway pressure, gram-negative infections and cigarette smoking have all been suggested as contributing factors (24).

Therapy

ARDS can be treated by mechanical ventilation and by several drugs. Besides treatment of ARDS, underlying and concomitant diseases should also be treated if possible. In patients with ARDS mechanical ventilation with positive end-expiratory pressure (PEEP) and high inspiratory oxygen fractions has usually to be applied. PEEP permits the arterial oxygen tension to be normalized at a lower inspiratory oxygen fraction, thus reducing the chance of oxygen toxicity. Effects of PEEP are mentioned later in this chapter. Extracorporeal carbon dioxide removal with low pressure and low frequency ventilation has been suggested as respiratory support. An advantage of this technique is that the injured lungs are relieved of the burden of a high F_{IO2} and peak airway pressure (8,56,80). This respiratory support is not commonly used in clinical practice.

The use of steroids has been advocated, but was not found to be effective in early stages (6). Based on the understanding of the pathophysiology of ARDS, the effects of cyclo-oxygenase inhibitors, anti-bodies against endotoxine, C5a and TNF, PAF antagonists and surfactant replacement have been investigated (1,58,61,103,116). Further studies are needed to evaluate the effects of these

drugs in ARDS patients.

Infant respiratory distress syndrome

Infant respiratory distress syndrome (IRDS) is a developmental disease of premature infants. Another name for IRDS is hyaline membrane disease. The incidence of IRDS in the United States was estimated to be about 40,000 infants of which 12,000 die annually (28). In the Netherlands 127 infants died from IRDS in 1989. The incidence ranges from 60 % in infants of up to 29 weeks gestation and declines with maturation to 20 % at 32 to 36 weeks and about 5 % in infants of 37 weeks and older (122). IRDS is more often found if the mother has diabetes, systemic hypertension or is a narcotics addict (122).

Clinically, IRDS patients are cyanotic at room air and show rapid breathing immediately after birth.

Pathophysiology

In the morphologic development of the fetal lung there are three stages: a glandular phase, during which the bronchial divisions are made; a canalicular phase of vascularization and further branching of the bronchial system; and the alveolar phase, during which definite alveoli are differentiated. In addition to morphologic differentiation and development, metabolic maturation also occurs in the lung. The fetal lung secretes fluid into the amniotic cavity and is also the site of surfactant synthesis. Surfactant appears in the fetal lung as early as the 16^{th} week, and its production increases with gestational age (10,92,125). Shortage of surfactant in the lungs causes an increased tendency of the alveoli to collapse at the end of expiration. In these infants IRDS develops. Lung volume and compliance are decreased causing hypoxemia and pulmonary hypertension (21,39,91). Microscopical findings in IRDS are: alveolar collapse with overdistension of dilated alveolar ducts, and pink-staining (hyaline) membranes on alveolar ducts (29). The muscular coat of pulmonary arteries is thickened, whereas the lumen is narrow. Lymphatic vessels are distended. Electron-microscopically alveolar cells appear damaged, lamellar bodies have disappeared and endothelial cells are swollen (29).

Therapy and outcome of IRDS

Therapy of IRDS consists of mechanical ventilation with high inspiratory oxygen fractions and PEEP. High-frequency ventilation can also be used, but some controversy remains on the application of this ventilatory mode (86,87). Surfactant replacement therapy is a recent development (25).

If IRDS is not complicated by additional disorders, recovery starts after 48 h. Usually oxygen therapy can be discontinued after 1 week. Infants with a very low birth weight will require a longer period of mechanical ventilation. Early complications are pneumothorax and pneumomediastinum. Bronchopulmonary dysplasia (BPD) is a late complication of IRDS. The incidence of BPD varies from 13 % to 75 % depending on birth weight (4). Prolonged exposure to high inspiratory oxygen fractions and high peak airway pressures are most frequently associated with BPD (20,88,108).

PEEP

A positive end-expiratory pressure (PEEP) is commonly used in ARDS and IRDS patients. PEEP is the maintenance of a pressure in the lungs greater than atmospheric at the end of expiration by applying such a pressure at the airway opening. The basic concept was proposed as early as in the 1930s by Barach et al. and Fulton and Oxon (5,32). PEEP was not used in clinical practice until the late 1960s (3). In pediatric patients PEEP was introduced by Gregory et al. (38). The beneficial effect of PEEP is an improvement of gas exchange (3). Mainly in the early phase of the respiratory distress syndrome, PEEP results in an increase in lung volume and alveolar recruitment, causing a rise in P_{aO2} and S_{aO2} (13,16,34,47,123).

PEEP, however, has negative effects on cardiac output by an increase in central venous pressure and lung stretch reflexes. These negative effects are partly compensated for by baroreflexes (17,18,40,98,99). A reduction in cardiac output negatively affects oxygen transport to the tissues.

An optimal approach of PEEP application has been matter of controversy. Some authors have advocated the use of high levels of PEEP, called "super-PEEP" (54). Barotrauma, which is frequently caused by a high peak and mean airway pressure, is a limiting factor for this approach (15,41,82).

Early use of PEEP in patients at risk for ARDS appears not to have any effects on the occurrence of ARDS or associated complications in most patients (79). An exception may be the patient group with multiple traumata, in which treatment with early osteosynthesis and early application of PEEP decreases mortality (37).

Suter et al. (110) defined "best PEEP" as the level, at which oxygen delivery to the tissues is maximal. He tried to use non-invasively measured variables to determine "best PEEP" and advocated respiratory sytem compliance as an indicator (110,111). Some authors doubted the value of compliance as an indicator for "best PEEP" (27,117). Venous admixture (33) as well as the

difference between arterial and end-tidal carbon dioxide tension have also been proposed as indicators for "best PEEP" (7,71). The differences in results regarding optimal PEEP approach may have been caused by different stages of ARDS, because in later stages end-expiratory lung volume can not be increased by PEEP (34). Another explanation for the differences in effects of PEEP could be whether or not fluid loading has been given in order to compensate for a decrease in cardiac output.

The minimal PEEP level required to maintain P_{aO2} above 60 mmHg at an F_{IO2} of 0.5 was reported by Carroll et al. (12) to be the best PEEP level. This was also found in other studies (47,128). The general approach used in clinical practice is based on this concept. A stepwise increase in PEEP is mostly applied with monitoring of oxygen transport until the inspiratory oxygen fraction can be decreased (85).

Animal models of ARDS and IRDS

ARDS arises from a large number of causes. A problem to study ARDS systematically in patients is that the clinical situation is usually different. To study certain aspects of ARDS, reproducible lung injury with predictable quality and quantity can be induced in animals. An extensive list of papers exists on a variety of models. Some essential papers will be mentioned in this chapter. Literature on the models, which are presented in this thesis, is discussed in more detail in the corresponding chapters.

Animal models of acute respiratory failure are characterized mainly by inflammation, edema or impairment of surfactant or a combination of these. Models can be divided into those that primarily act on the capillary endothelial side of the lung and those that primarily act on the alveolar side. Usually a combination of endothelial and epithelial damage is present.

Numerous models mainly act on the endothelial side of the lung.

Sepsis can be induced in animals by intravenous administration of Pseudomonas aeruginosa, Escherichia coli or Staphylococcus aureus (11,31,48,74). In the lung this results in inflammation, permeability edema and pulmonary hypertension probably mediated by PMN's (11,31,48,74). Cardiovascular function and lung vasculature are mostly impaired (31,74).

Many of the substances, which are involved in the pathogenesis of ARDS and sepsis, can induce lung injury in animals.

Endotoxin administration results in an inflammatory reaction and edema with an increase in pulmonary arterial pressure (68). Cardiovascular failure often develops (70,120). The endotoxin model shows large interspecies variation.

After endotoxin administration a rise in leukotrienes (LT) occurs. Also intravenous injection with *LTD4* results in edema. These results indicate that this substance could be important in the pathogenesis of endotoxin injury (101).

TNF, which was suggested as a primary mediator in endotoxin shock, results in similar lung injury as endotoxin (51).

Intravenous infusion of *PAF* increases pulmonary arterial pressure (103). PAF in combination with endotoxin results in a marked increase in TNF, accumulation of granulocytes in the lungs, lung edema and systemic hypotension (89).

Intraperitoneal administration of *zymosan* leads to activation of complement and inflammation, and edema in the lungs (14,36).

Injection of *oleic acid* produces edema and pulmonary hypertension by direct toxic effects on endothelial lining and an inflammatory reaction (41,49).

Alpha-naphtylthiourea (ANTU) and *nitrous oxide* result in permeability edema (69,124).

Injection of *muscle cubes* causes a diffuse obstruction of pulmonary arteries, aggregation of platelets, release of mediators and pulmonary hypertension (113).

Intravenous injection of *phorbol myristate* acetate causes endothelial damage and activation of inflammatory cells resulting in both acute lung injury and systemic organ failure (50,102).

Damage of the lung by administration of *paraquat* is probably mediated by oxygen-derived free radicals. Depending on the route of administration paraquat primarily acts on the alveolar or on the endothelial side (55).

Models that primarily act on the alveolar side of the lung can be induced in several ways.

Aspiration of *hydrochloric acid* solution causes pulmonary edema by damage to the epithelium and surfactant (59), while eicosanoids may be important in the pathogenesis (73).

Exposition to *pure oxygen* results in respiratory failure by surfactant dysfunction, inflammation and increased lung permeability (26).

Repetitive total *lung lavages* with saline result in atelectasis, edema and pulmonary hypertension by surfactant deficiency and mediators from granulocytes (53,57).

Surfactant dysfunction can also be induced by *N-Nitrososo-N-methylurethane* (NNNMU). This substance causes an increase in protein permeability and an impairment of gas exchange. Pool size of surfactant is decreased and phospholipid composition is altered (62,95).

IRDS can be studied in *premature animals*. Hyaline membranes are a common finding. In premature rats and rabbits functional studies are limited due to the size of the animals. Premature lambs, which are of a satisfactory size to

insert catheters and flow meters, have another disadvantage. In these animals the degree of lung maturity has a large variability (67,76,105).

Outline of the thesis

So far the existing animal models have a lack of long lasting stability. Hardly any attention was paid to standardization of the induction of respiratory distress. We aimed at models in which the individual animals have a comparable respiratory distress for several hours to obtain the opportunity of comparative studies on interventions. We have chosen for two models in pigs: one model implied damage of the alveolar part of the alveolar-capillary membrane, and the other implied damage of the endothelial part. The first model was induced with alveolar lavage as an analogy of primary depletion of surfactant; the second was induced by intravenous administration of oleic acid as an analogy of fat embolism.

We developed both models under guidance of the most commonly used criteria of ARDS (72,84; Table 1.1).

The lavage model is described in the chapters II to V. In chapter II literature on the lavage model is reviewed. The development and the pathophysiological characteristics of our lavage model are presented in chapter III. In chapter IV the nature and distribution of pulmonary vasoconstriction in lavage induced respiratory distress is analyzed by morphometry. Chapter V contains the hemodynamic and gas exchange effects of PEEP in the lavage model.

The oleic acid model is described in chapters VI to IX with the same order of chapters as the lavage model: a literature review in chapter VI, the development of the model in chapter VII, the morphometry of the muscular pulmonary arteries in chapter VIII and the PEEP study in chapter IX.

In chapter X the results of our studies with the lavage and oleic acid model are compared and considered.

The thesis is summarized in chapters XI and XII in English and Dutch language respectively.

Chapters III to V and VII to IX are submitted for publication as independent papers, implying an overlap in method description. Parts in method description, which overlap with a former chapter are printed in smaller characters.

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CHAPTER II

EXPERIMENTAL LAVAGE MODELS

Impairment of lung surfactant is involved in the pathogenesis of adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS) (3,4,31,52,69,83). Near-drowning as an origin of ARDS is most markedly associated with surfactant deficiency and fluid overload.

Near-drowning

Drowning causes about 140,000 deaths a year in the world, of which about 9000 occur in the United States of America (15,66). In the Netherlands 361 cases of death by drowning occurred in 1989. The number of near-drownings, i.e. survival rate after immersion in water, is estimated to be about nine times that of mortality (66). About 50% of the victims is less than 20 years old. Survival rate after optimal treatment of near-drowning is about 80% (16). However, if severe cerebral damage develops mortality rate can be up to 100% due to hypoxemia and edema (15). Hypothermia can sometimes prevent neurological damage (85). Ten to twelve percent of drowning victims do not actally aspirate water, but die instead from reflective laringospasm leading to hypoxemia, "dry drowning". The other 90 % aspirate freshwater or seawater, which both result in a severe hypoxemia (14). As seawater is hypertonic fluid is drawn from the circulation into the lung, resulting in an increase in venous admixture (56). Aspirated freshwater is rapidly absorbed from the lung into the circulation (80). Freshwater alters surface properties of surfactant resulting in a decrease in alveolar ventilation (28). Both aspiration of seawater and aspiration of freshwater cause pulmonary edema (57). Lung injury can also result from micro-organisms or substances in the aspirated fluid (35). In patients that temporarily survived near-drowning a bronchopneumonia and hyaline membranes are often seen on microscopical examination (60).

Cardiovascular changes can be due to hypothermia, hypoxemia, acidosis or fluid overload. About 15% of nearly-drowned victims aspirate sufficient water to result in a significant fluid overload (58). Hypoxemia can result in ventricular fibrillation (80). Central venous pressure increases immediately after aspiration of small quantities of freshwater and seawater, but rapidly returns to normal values (56). Aspiration of large quantities of freshwater results in an increase in central

venous pressure, followed after about 1 h by a recovery to normal values. Aspiration of a large amount of seawater causes an initial, but transient rise in central venous pressure, followed by a rapid decrease. This is probably due to a decrease in circulating blood volume. Bradycardia, which is sometimes found, may be the result of a diving reflex (35).

Therapy of nearly-drowned victims consists of immediate cardiopulmonary resuscitation at the site of the accident. After evaluation of the patient's condition mechanical ventilation with PEEP and high inspiratory oxygen fraction is commonly applied. This usually results in an increase in arterial oxygen tension (8,22). Metabolic acidosis has to be corrected (59).

Hypoxemia, which predominantly results from surfactant impairment and edema, is the most important cause of morbidity and mortality.

Surfactant

Function

In 1929 Von Neergaard (62) reported that part of the recoil forces of the lungs depends on a surface tension acting at the air-fluid interface of the alveolar lining fluid. Surface tension at an air-fluid interface produces forces, which tend to reduce the area of the interface. In the alveolar lining fluid surface tension is reduced by substances, which are called surfactant. A reduction of surface tension promotes lung expansion on inspiration and prevents lung collapse on expiration (13). Surfactant also reduces the transsudation of fluid into the alveolar spaces (1,13).

Composition

Lung surfactant is mainly composed of phospholipids and proteins. The phospholipids consist of a hydrophobic side, which projects into the alveolar gas, and a hydrophylic side which projects into the alveolar fluid. These characteristics of surfactant result in a layer of phospholipids on the alveolar lining.

The protein content of surfactant varies considerably. The most abundant apoprotein of surfactant is protein A, SP-A. Other proteins are SP-B, SP-C and SP-D. These four surfactant-associated proteins are important for the movement of phospholipid from lamellar structures to the alveolar surface film. SP-A probably regulates the turnover of alveolar surfactant (47). SP-A and SP-B are important for the transformation of the multilayered membranes of lamellar bodies into the complex three-dimensional lattice of the tubular myelin structure, that develops from secreted surfactant (79). SP-B and SP-C accelerate the formation rate of phospholipid films at the air-fluid interface (33).

Production and metabolism

Surfactant is produced in the lung by the pneumocyte cells type II (Figure 2.1). About 15 % of the cells in the distal lung are pneumocytes II. The ultrastructure is similar in most animal species (18,30). The time after injection of radiolabeled phospholipids to reach maximal activity varied between animal species from about 5 to 10 h in rats and sheep respectively (7,40). This could be an indication that production time varies between animal species.

After production surfactant is stored in specialized secretorial organnels of the pneumocytes II, named lamellar bodies. Pneumocytes II contain approximately 100-150 lamellar bodies per cell (86). The surfactant phospholipids in the lamellar bodies are layered and surrounded by a membrane. The lamellar bodies also contain lysosomal enzymes and a variety of proteins (29). Only a small reserve of surfactant phospholipids is present in the intracellular pool (86). Under resting conditions about 10% of phospholipids in the lamellar bodies is secreted per hour into the lumen of the alveoli (86). Surfactant secretion can be stimulated by adrenergic stimulation (67), lung inflation (34), hyperventilation (63) and prostaglandines (67).

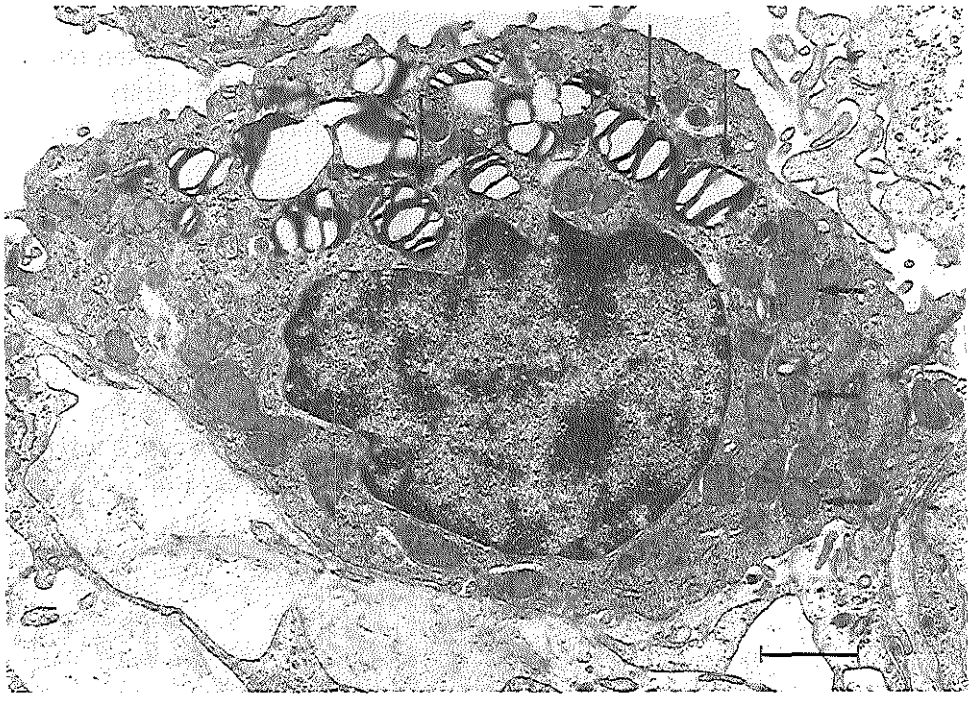

Fig. 2.1. Pneumocyte II lying on thin basement membrane. In the abundant cytoplasm there are numerous lamellar bodies (large arrows) and mitochondria (short arrows).

Clearance of surfactant from the alveoli occurs mostly by reuptake and reutilization of the surfactant lipids after degradation by pneumocytes II and alveolar macrophages (84).

Mechanical ventilation may negatively affect surfactant function (25,26). Ennema et al. (23,24) found an inactivation of surfactant by positive end-expiratory pressure in normal rabbits.

Lavage model

History

In patients total lung lavages with 0.9 % saline are done as a therapy of alveolar proteinosis (12,73). The first lavage models in animals were studied to analyze effects of lavages for this purpose (27,37,38). In 1966 Huber et al. (37) described the effects of successive total lung lavages with 0.9 % saline in dogs. These authors reported that lavages removed surface active substances, which consisted of lipids and proteins and resembled alveolar lining material. This was confirmed by Finley (27) and Levitsky (54). Kelly et al. (43) concluded from studies in humans with segmental broncho-alveolar lavages that besides a flux of fluid from the alveoli into the circulation, also a flux from the circulation into the lungs existed with a net flux into the lungs indicating formation of edema.

Instillation of fluid into the lungs was used as a model of drowning (28,65). Giammona et al. (28), who studied drowning in dogs, concluded that saline and sea water were able to wash-out surfactant, whereas distilled water resulted in less wash-out of surfactant. Sea water resulted in less fluid absorption than distilled water. Instillation of distilled water caused death by ventricular fibrillation due to volume overload. For distilled water Qualls et al. (71) found a resorption rate from the alveoli of 50% in 4 min, whereas 0.9 % saline had a resorption rate of 50% in 15 min if 6 ml fluid per kg body weight was instilled into the airways of dogs. Orlowski et al. (65) concluded that distilled water affected gas exchange more than 0.2, 0.9, 2 and 3 % saline solutions. Regardless of tonicity of the instilled fluid the animals in this study developed a severe bradycardia and a low cardiac output.

In more recent years Lachmann et al. (48,49) introduced total lung lavages with 0.9 % saline in animals to develop a model of respiratory distress. The lavage regime consisted of about ten lung lavages at 5 min intervals in mechanically ventilated guinea-pigs. This model has been used by many authors as mentioned in chapter III to study features of acute respiratory distress in animals.

Pathophysiology of the lavage model

Surfactant impairment

Total lung lavages result in a progressive depletion of surfactant phospholipids (6,24,49). As a result local to widespread atelectasis is found (49,51,55). Because synthesis remains low, a depletion of surfactant occurs after lavages (24). Lachmann et al. (48) concluded that stored phospholipids were released into the alveolar spaces, but that the short interval of 5 min between the lavages did not permit renewal of the tissue pool. The phospholipid composition of recovered surfactant changes by lavages (6,24). Continuous positive pressure ventilation causes a decrease in lamellar body fraction of lung tissue (24). Surfactant replacement therapy, given shortly after lavage, increases P_{aO2} and decreases P_{aCO2} (6,24,44).

These studies indicate that surfactant depletion is important for the pathogenesis of lung injury after lavage.

Additional factors

A diffuse pneumonitis with the appearance of polymorphonuclear granulocytes has been found after lavage (9,32,37,42,49). Kawano et al. (42) reported that rabbits with normal cellular composition of the blood had a poor gas exchange, a substantial protein leakage into the lung and extensive hyaline membranes, whereas rabbits depleted of granulocytes by pretreatment with nitrogen mustard had less disturbances of the lungs. Repletion of granulocytes led to poor gas exchange and hyaline membrane formation in these animals. Thus, besides surfactant depletion granulocytes appear to be important for the pathogenesis of the lavage model.

Hyaline membranes and epithelal damage with necrotic cells have often been found in guinea-pigs (49), rabbits (32,42,45,55,72) and dogs (50). Epithelial lesions are more extensive in rabbits after 7 h of ventilation than immediately after lavage. This indicates that a period of mechanical ventilation leads to further damage to lungs, that were previously injured by lavage (55). In accordance with this are the findings from studies with high-frequency ventilation and extra-corporeal membrane oxygenation. These modes of ventilation result in a better oxygenation and CO_2 elimination and less hyaline membranes compared with conventional mechanical ventilation (21,24,32,39,41,45,64,72,78,87).

Most studies were done with an inspiratory oxygen fraction of 1.0, which is potentially toxic for lung tissue and could have contributed to lung damage.

Gas exchange and pulmonary mechanics

After lavage arterial oxygen tension is lowered and arterial carbon dioxide

tension is increased. Functional residual capacity and lung compliance are decreased (49). After about eight lavages in LEWI-minipigs Dauberschmidt et al. (20) found a large interindividual variation in P_{aO2} and lung compliance. Nielsen et al. (64) performed ten to twelve lung lavages to obtain RDS in pigs. These authors tested the stability of their lavage model for brief periods with a standard ventilation in between different ventilatory modes. From these data they concluded that their lavage model was stable after lavage. Extra-vascular lung water was increased after lavage compared to the baseline conditions before lavage. It remained constant throughout the experiments (9,64,75).

The effects of lavage have usually been studied under conditions of pressure controlled ventilation. Because lung compliance is decreased after lavage, pressure controlled ventilation will have led to a decrease in tidal volume, resulting in a decreased alveolar ventilation (82). Therefore, gas exchange may have been affected not only by the lung lavages, but also by changed ventilatory conditions. Other ventilatory conditions, such as PEEP and frequency of ventilation, have also been changed occasionally after lavages (55,72). Stability over time with respect to gas exchange and hemodynamics without any interventions has seldom been evaluated in literature.

Hemodynamics

Systemic arterial pressure and cardiac output have been found to be either the same as before lavage (17,72) or decreased significantly (11,87). Pulmonary arterial pressure usually increases by lavage (9,39,72). Burger et al. (9) supposed that post-lavage pulmonary hypertension was mediated by thromboxane A2.

Mortality

To induce respiratory distress repetitive lavages were done, which often resulted in a high mortality rate (17,44,46,72). Death of the animals was caused by pneumothorax, circulatory overload and hemodilution (17,28,55,64,71,72). Nielsen et al. (64) reported that the lavages sometimes caused a circulatory instability, which was attributed to the high intra-thoracic pressure during a lavage. Therefore, the lavage procedures were sometimes interrupted. No sytematic study has been done to decrease mortality. Mortality may be reduced by using a minimal number of lavages.

PEEP

Positive end-expiratory pressure (PEEP) is a commonly used therapy in ARDS and IRDS. An important effect of PEEP is recruitment of alveoli resulting

in an increase in end-expiratory volume (V_{EE}) and P_{aO2}. Effects of PEEP in patients are described in more detail in chapter I.

The inflection point (P_{flex}) is the pressure on the pressure-volume curve of the respiratory system, where the slope increases. The pressure-volume curve of the respiratory system is usually obtained by stepwise inflation of the lungs. After lavages the effects of PEEP have mostly been studied at two levels of PEEP being low PEEP and PEEP above P_{flex} (2,11,68,74). High PEEP increases P_{aO2} and V_{EE} and results in less hyaline membranes compared to low PEEP (2,11,50,51,68,74). P_{flex} is supposed to indicate recruitment of alveoli. However, the importance of P_{flex} was disputed by Pesenti et al. (68), who found that mean airway pressure was a more important determinant of gas exchange than a PEEP level above or below P_{flex}. Most authors found a decrease in systemic arterial pressure and cardiac output at high PEEP (11,50,68,74).

Objectives

In most studies on effects of lavage, ventilatory settings were often changed. Hardly any data are available to evaluate the stability of the animal's condition after lavage (50). "Best PEEP", which is defined as the level of PEEP where oxygen delivery is maximal, has not been studied after lavage.

We aimed to develop a model of respiratory distress by lavages in pigs with volume controlled ventilation and an inspiratory oxygen percentage of 60%,

- which fulfills the clinical criteria by Petty (70) and Murray (61),
- which has a low mortality rate and
- which is stable for several hours to study interventions.

In the model the characteristic changes in gas exchange, hemodynamics and morphology are analyzed. As an intervention the effects of PEEP to find indicators of "best PEEP" are studied.

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CHAPTER III

EVALUATION OF A LAVAGE REGIME TO OBTAIN A STABLE MODEL OF RESPIRATORY DISTRESS IN PIGS

To wash out surfactant in order to interfere with alveolar gas exchange a regime of repetitive lung lavages, up to ten or more, has often been used (10,13,16,22,24,26,32, 33, 36). No systematic study was done to analyze the effects of each successive lavage on gas exchange and circulation. Hardly any attention was paid to the development of a respiratory distress model satisfactory stable for several hours.

Several studies were done with pressure controlled ventilation leading to a decrease in tidal volume and alveolar ventilation due to a decrease in lung compliance after the lavages (13,26,36). In other studies ventilation was regulated to maintain arterial carbon dioxide at baseline level (10,16,33). In some studies positive end-expiratory pressure was adjusted to maintain normocapnia (24,32). In all these studies gas exchange was not only affected by the lavages, but also by changes in ventilatory settings (39). To eliminate the effect of ventilation we used constant ventilatory parameters to study the responses of the respiratory system and the circulation to successive lavages. These responses were analyzed in different lavage protocols. Using the response of arterial oxygen tension as a main guide we selected an optimal regime of lavages causing a stable respiratory distress in pigs.

We aimed at a stable period of about five hours in order to obtain an animal model for studies on basic mechanisms and therapeutic interventions in early respiratory distress.

METHODS

Surgical procedures and ventilatory conditions

Twenty three young pigs (5-7 weeks old, 9.6 $\pm$ 0.8, sd, kg body weight) were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 $mg.kg^{-1}$, kg^{-1} refers to body weight) and placed in supine position on a thermo-controlled operation table to maintain body temperature. Anesthesia was

maintained by a continuous infusion of sodium pentobarbital (8.5 $mg.kg^{-1}.h^{-1}$). After the surgical procedures, tubocurarine (0.2 $mg.kg^{-1}.h^{-1}$) was given to suppress spontaneous breathing. After tracheostomy the pigs were connected to a volume controlled ventilator.

A polythene single lumen catheter was inserted through the right common carotid artery into the aortic arch for measuring arterial blood pressure and sampling of blood. Three catheters were inserted via the right external jugular vein: 1) a Swan-Ganz catheter into the left pulmonary artery to monitor pulmonary arterial pressure and pulmonary blood temperature and to sample mixed venous blood; 2) a double walled catheter into the right atrium for injection of saline at room temperature during the thermodilution procedures, and 3) a four lumen catheter into the superior vena cava to measure central venous pressure and for infusion of fluids and anesthetics. The pressure catheters were continuously flushed at a flow rate of 3 $ml.h^{-1}$ with normal saline containing 10 I.U. per ml heparine to avoid clotting in the catheters. In total 9 ml fluid and 90 I.U. heparine were infused per hour. A catheter was put into the urinary bladder to avoid retention of urine.

After surgery the animals were connected to a computer controlled ventilator (20), containing two separate bellows. One bellows for continuous ventilation throughout the experiments, the other for alternative ventilation during the special procedures of lung volume estimations. Tidal volume (V_T) was adjusted to a P_{aCO2} of 38-42 mmHg during baseline. A ventilatory frequency of 10 breaths per minute was used. An inspiratory fraction of oxygen (F_{IO2}) of 0.6 and a positive end-expiratory pressure (PEEP) of 2 cmH_2O were applied throughout all experiments. The ratio of inspiration: expiration was 2:3. These ventilatory parameters were kept constant during the experiments.

Measured and estimated data.

Gas exchange, acid-base and hemoglobin

Oxygen and carbon dioxide tensions and the acid-base indices were determined with use of an automatic blood gas analyzer (Radiometer ABL3). Values were calculated for standard bicarbonate (HCO_3^-). Hemoglobin concentration (Hb) and oxygen saturation were determined with use of an oxymeter (Radiometer OSM2). All blood samples were replaced by an equal volume of saline. Inspiratory and mixed expiratory gases, including Helium, were analyzed by a mass spectrometer (Perkin-Elmer, MGA 1100).

Venous admixture (Q'_s/Q'_t) was calculated in percentage of cardiac output (Q'_t) according to (5):

$$Q'_s/Q'_t = 100 \times (C_{cO2}-C_{aO2})/ (C_{cO2}-C_{\bar{v}O2})$$

Oxygen content in arterial (C_{aO2}), mixed venous ($C_{\bar{v}O2}$) and pulmonary end-capillary blood (C_{cO2}) was calculated according to the equation:

$$C_{xO2} = (S_{xO2} \times 1.39Hb) / 100 + (0.0031P_{xO2})$$

where subscript x stands for a, $\bar{v}$ and c respectively. S_{xO2} is oxygen saturation in percentage and P_{xO2} oxygen tension in mmHg. Oxygen binding capacity, 1.39 ml oxygen per g Hb, was used as prescribed by the International Committee for Standardisation in Haematology in 1965. The solubility of oxygen, 0.0031, was expressed in ml per 100 ml blood and per mmHg. C_{xO2} was obtained in ml O_2 per 100 ml blood.

The saturation of the pulmonary end-capillary blood (S_{cO2}) was derived from the oxygen saturation curve of pig blood and the alveolar oxygen tension (P_{AO2}) as an estimate of the pulmonary end-capillary tension. The parameters in the equation of the human oxygen saturation curve (23) were fitted for the pig's oxygen saturation curve, based on pig blood data from other experiments in our laboratory. This oxygen saturation curve was similar to the curve described by Bartels (4).

P_{AO2} was derived from the equation (2):

$$P_{AO2} = P_{IO2} - P_{ACO2} \times [F_{IO2} + (1 - F_{IO2}) / R]$$

where P_{IO2} and F_{IO2} are the inspiratory oxygen tension and fraction respectively. P_{ACO2} is the alveolar carbon dioxide tension, which we assumed to be equal to P_{aCO2}, and R is the respiratory quotient.

Physiological dead space (V_D) in percentage of V_T was obtained from the equation (14):

$$V_D/V_T = 100 \times (P_{aCO2} - P_{\bar{E}CO2}) / P_{aCO2}$$

where $P_{\bar{E}CO2}$ is the mixed expiratory carbon dioxide tension.

Pulmonary data

End-expiratory lung volume

To estimate end-expiratory volume (V_{EE}) of the lungs we used a closed helium dilution method. Ventilation was switched from one bellows of the computer controlled ventilator to an in parallel functioning bellows which was flushed before with helium at a concentration of about 4% (He_{b2}). Rebreathing of

the helium was started at the end of an expiration. When the helium concentration attained an equilibrium (He_{eq}) ventilation was switched back, again at end-expiration. V_{EE} was calculated from the mass balance:

$$(V_{EE} + V_{tub} + V_{b2}) \times He_{eq} = V_{b2} \times He_{b2}$$

where V_{tub} is the volume of the tubes, connecting ventilator and animal, and V_{b2} is the volume of the bellows containing helium. Corrections were made for volume and helium loss due to the sample flow to the mass spectrometer. An additional correction for volume loss due to gas exchange was made by simultaneously measuring nitrogen (N_2) concentrations, assuming a constant amount of N_2.

Total respiratory compliance

The compliance of lungs and thorax (C_{rs}) was estimated with use of an inspiratory pause method (7,37). Tracheal pressure (P_T) was measured in the cannula (gas pressure transducer, type 270, Hewlett Packard). Three inspiratory pauses of 14.5 sec with insufflation volumes of 6, 12 and 18 $ml.kg^{-1}$ were inserted at intervals of two min during normal ventilation. During these pauses tracheal pressure and thoracic volume decreased gradually. These volume changes during the maneuvers were recorded with use of a mercury cord, which was fixed around the thorax about 5 cm cranial from the sternal xyphoid. The three inflation volumes, mentioned above, served to calibrate the mercury cord. To estimate C_{rs} a third degree polynomial pressure-volume (P-V) curve was fitted through end-expiratory pressure and volume, and the pressures corresponding with the three end-inspiratory volumes. The relationship yielded an approximately linear part between the volumes 4 and 8 $ml.kg^{-1}$. Compliance was derived from this part of the P-V curve (17). Compliance estimates were obtained during baseline conditions and at the end of the experiments. Estimates of C_{rs} before and after the lavages were compared at the same thoracic volume level.

During ten hours the mercury cord was tested at room temperature for its stability by stretching and releasing it at a rate of 10 per minute and an amount of stretch corresponding to that during mechanical ventilation. We did not find any change in zero level and gain.

Weight of lungs and heart and morphology

At the end of the experiments, the animals were killed with a high dose of sodium pentobarbital (0.07 $g.kg^{-1}$). Immediately after death the lungs were fixed by instillation of formalin through the trachea. After ligation of the blood vessels

heart and lungs were removed and weighed. The amount of instilled formalin was subtracted from this weight. Blocks of tissue were taken from apical and diaphragmatic lobes at either side. From these blocks histologic slides were cut and stained with hematoxylin and eosin and with an elastic-van Gieson stain.

In some experiments blocks for electron microscopic examination were taken from the middle lobe of the lung and immediately fixed in glutaraldehyde. The blocks were postfixed in osmium, dehydrated in acetone, embedded in LX112 and stained with uranyl acetate and lead citrate.

Hemodynamic data and oxygen delivery

Arterial blood pressure (P_{ao}), pulmonary arterial blood pressure (P_{pa}) and central venous blood pressure (P_{cv}) were measured continuously with use of Statham transducers, type P23De. Pressures were referred to ambient air pressure and to a zero level at the height of the manubrium. Pulmonary capillary wedge pressure (P_{pcw}) was measured intermittently. All pressures are presented as mean values over a ventilatory cycle.

Cardiac output (Q'_t) was determined by the thermodilution method. Four determinations were equally spread over the ventilatory cycle and were performed in four minutes. The average was used as an estimate of Q'_t (18,19).

Oxygen delivery (D_{O2}) was calculated according to:

$$D_{O2} = C_{aO2} \times Q'_t$$

Experimental procedures and data acquisition

Protocol of the experiments.

After the surgical procedures a stabilisation period of about half an hour followed. In this period V_{EE} was determined and chest X-rays during inspiration were made. Next, the baseline data of gas exchange and circulation were determined three times during one hour from -1 to 0 h, where 0 h is the time of the first lavage. C_{rs} was obtained only once in the last half hour. After the baseline period a series of lavages was performed, followed by the determination of gas exchange and hemodynamic variables shortly after each lavage. At the end of the experiments V_{EE}, C_{rs} and chest X-ray images were obtained again.

The lavage procedure.

A lavage procedure was performed with 35 $ml.kg^{-1}$ 0.9% saline via a side tube of the tracheal cannula. Inflow of saline into the lungs took 20 seconds and

outflow 15 seconds, immediately followed by the same sequence with the same fluid. The position of the reservoir was standardized at 55 cm above and 50 cm below the manubrium. A lavage procedure started from end-expiratory volume and lasted 90 s, including all additional manipulations.

Lavage regimes

In a first group of pigs (group I, n=3) the effects of a lavage procedure (lavage 1) were studied. When after recovery a new steady state in P_{aO2} was observed for at least half an hour, another lavage procedure was performed (lavage 2). Again the effects were recorded until a new steady state in P_{aO2} was established. We continued this protocol until a low and stable P_{aO2} (about 50 mmHg) without recovery was obtained.

In group II (n=3) the protocol of group I was repeated but with application of two lavages (lavage 1 and 2) at an interval of 5 minutes. After recovery of P_{aO2} followed by a new steady state, another pair of lavages (lavage 3 and 4) was performed.

In group III (n=8) the lungs were lavaged according to the protocol of group II but with the second pair of lavages one hour after the first pair.

In group IV (n=3) the interval between the two pairs of lavages was reduced to half an hour.

To compare the different lavage regimes on a similar time scale the first lavage was always set at 0 h.

Fluid balance

We calculated the fluid balance from the beginning of the baseline observations until the end of the experiments. This balance was corrected for an estimated loss of fluid by evaporation into the ventilatory air and via the skin. An additional correction was made for the production of water by metabolism. Details of these estimations are given in the legend of Table 3.2.

Control experiments

In healthy non-lavaged pigs (n=6) mechanical ventilation was performed for the same time as in the animals that received lung lavages. Sham lavages were done by discontinuing ventilation at end-expiration for 90 s, being the time of a lavage. The same protocol as that of group III was used.

Criteria of respiratory distress

We used the criterion of respiratory distress by Petty (30) as a guideline for the development of our lavage model: $P_{aO2} < 50$ mmHg, for $F_{IO2} \geq 0.6$. Murray suggested an expanded definition of respiratory distress (29) by applying a score system to characterize the presence and severity of the disease by the following features: chest roentgenogram score, hypoxemia score (P_{aO2}/F_{IO2}), positive end-expiratory pressure score and respiratory system compliance score. To determine the score of C_{rs} in our pigs of about 10 kg we recalculated Murray's values per kg body weight assuming an average weight of 70 kg in his patients. This score system was implied in the evaluation of our model.

Statistical analysis

The results were analyzed using t-tests for paired and unpaired samples when only two mean values were compared. To test the significance of changes in time in the post-lavage period we used standard repeated measures analyses of variance (SPSS-MANOVA). P-values < 0.05 were accepted as statistically significant. Data are presented as mean values $\pm$ 1 sd.

RESULTS

Control experiments

No changes were found in the majority of gas exchange, pulmonary and hemodynamic variables over 6 h after the sham lavages (Table 3.1). The decrease in P_{aO2} was already present at fifteen minutes after the last sham lavage. C_{rs} decreased about 12% over a 6 hour period. Oxygen delivery decreased 20% due to a corresponding decrease in cardiac output. Moreover, a slight decrease occurred in the standard HCO_3^- concentration (-3%). The fluid balance was 22.2 $ml.kg^{-1}$ positive over the 8 h observation period (Table 3.2).

Gas exchange, acid-base and hemoglobin

The first lavage in group I (n=3) decreased P_{aO2} in two of three animals

Table 3.1. Respiratory and hemodynamic data of the control group.

	baseline			7 h		
P_{aO2} (mmHg)	304	±	22	280	±	20 *
S_{aO2} (%)	100	±	1	100	±	1
P_{aCO2} (mmHg)	39.0	±	1.5	38.4	±	0.6
V_D/V_T (%)	28	±	11	30	±	5
Q'_s/Q'_t (%)	4	±	3	3	±	1
Hb (mmol.l^{-1})	6.2	±	1.0	6.5	±	1.0
pH	7.48	±	0.02	7.47	±	0.01
HCO_3^- (mmol.l^{-1})	29.2	±	1.0	28.4	±	0.2 *
V_{EE} (ml.kg^{-1})	21.0	±	1.5	20.8	±	0.8
C_{rs} (ml.cmH_2O^{-1}.kg^{-1})	1.7	±	0.2	1.5	±	0.3 *
Q'_t (ml.s^{-1}.kg^{-1})	2.2	±	0.4	1.7	±	0.3 *
D_{O2} (ml.s^{-1}.kg^{-1})	32.3	±	6.9	26.0	±	4.1 *
P_{ao} (mmHg)	92.7	±	23.3	86.0	±	23.1
P_{pa} (mmHg)	8.6	±	1.0	9.5	±	0.6
P_{cv} (mmHg)	-0.5	±	0.4	-0.9	±	0.6
$P_{T,p}$ (cmH_2O)	18	±	1	20	±	2

Mean values ± sd, n=6. Values at 7 h were tested versus their baseline values. * $p < 0.05$.

from 311 and 313 mmHg to 183 and 165 mmHg respectively in the first hour after the lavage (Fig. 3.1a). Two hours after the lavage P_{aO2} was recovered close to baseline values. In the third animal the first lavage hardly affected P_{aO2}. In all three animals P_{aO2} decreased sharply after the second lavage to 86.6 ± 14.7 mmHg and declined gradually to a minimum half an hour after this lavage. Then, P_{aO2} recovered to 113 mmHg in one experiment and to 231 and 204 mmHg in the other two. A third lavage was performed when P_{aO2} was stable again at about three hours after the second. The third lavage caused a stable low level of P_{aO2} in two animals at about 46 mmHg and in one at 72 mmHg. In this third experiment an F_{IO2} of 1.0 was applied for one hour, which increased P_{aO2} to about 130 mmHg. Then, F_{IO2} was lowered to 0.6 again and P_{aO2} was the same as before.

In group II (n=3) the first pair of lavages caused a sharp decrease in P_{aO2} (Fig. 3.1b) from 328 ± 21.6 mmHg to 62.0 ± 13.5 mmHg. P_{aO2} gradually recovered to a stable level of 275 ± 16 mmHg in 3-5 hours. After the second pair of lavages P_{aO2} fell sharply to 53.1 ± 1.1 mmHg and subsequently remained stable for 5-7 hours.

In group III (n=8) P_{aO2} decreased from 300 ± 25.0 mmHg to 59.8 ± 13.1

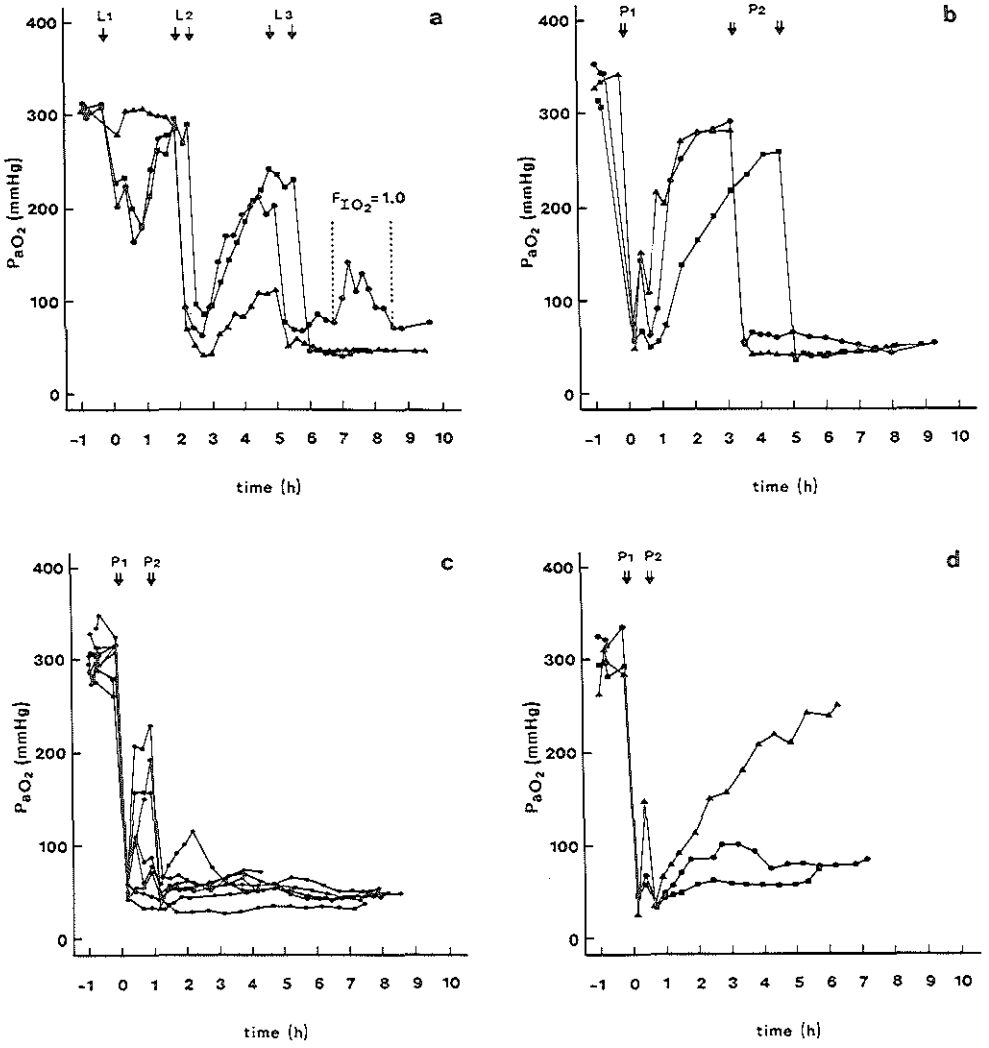

Fig. 3.1. Lavage regimes.

In all diagrams P_{aO2} is plotted against time, where zero-time corresponds to the moment of the first lavage. Baseline observations were done between -1 and 0 h. a. group I: L1, L2 and L3 indicate one lavage each. The two arrows at L2 and L3 respectively indicate the difference in time between the single lavages in the different animals. b. group II: P1 and P2 indicate a pair of lavages. P2 was done at different moments for the different experiments in a new steady state. c. group III: two pairs of lavages (P1 and P2) at an interval of one hour. d. group IV: two pairs of lavages at half an hour.

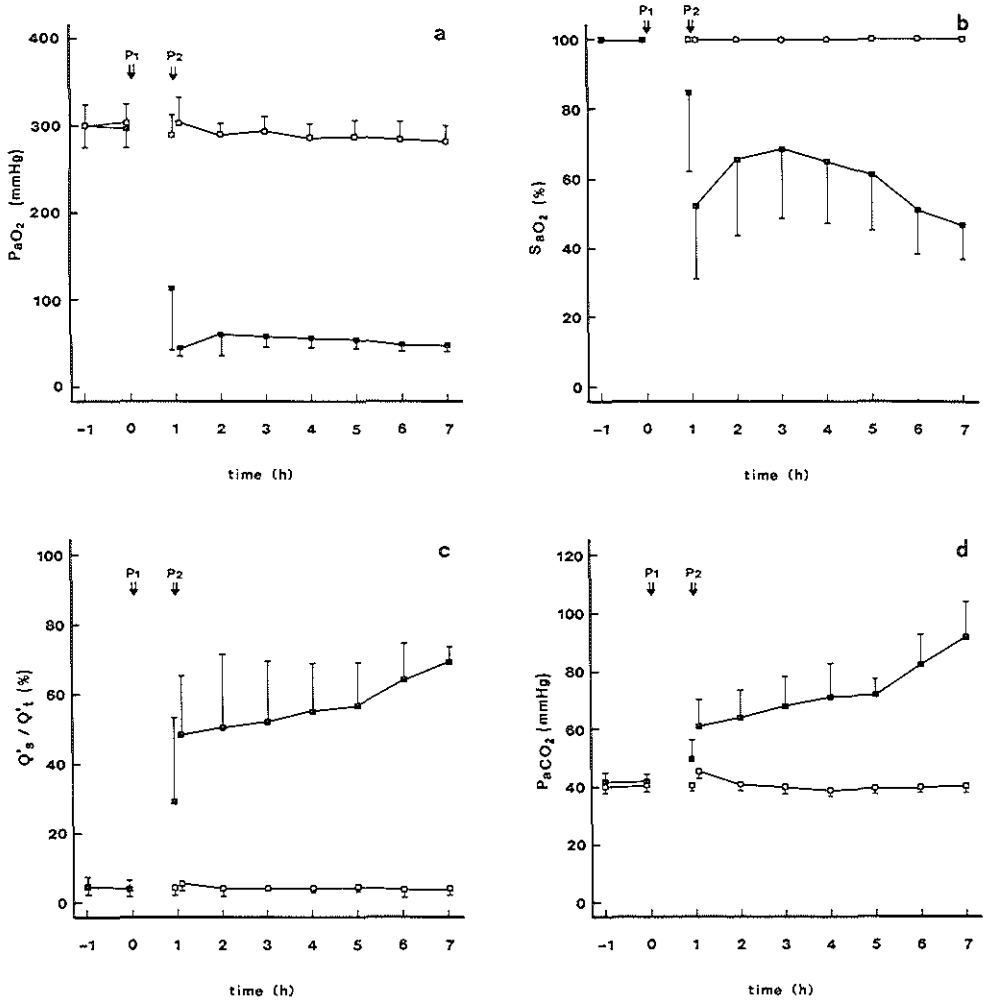

Fig. 3.2. Data of gas exchange.

Time scale as Fig. 3.1.c. Open squares: averaged values of control group (n=6). Closed squares: averaged values of lavage group (n=8, from 4 h: n=6), vertical bars: sd. a. arterial oxygen tension, b. arterial oxygen saturation, c. venous admixture, d. arterial carbon dioxide tension, e. pH, f. standard HCO_3^- concentration. Figs. 3.2e. and f.: next page.

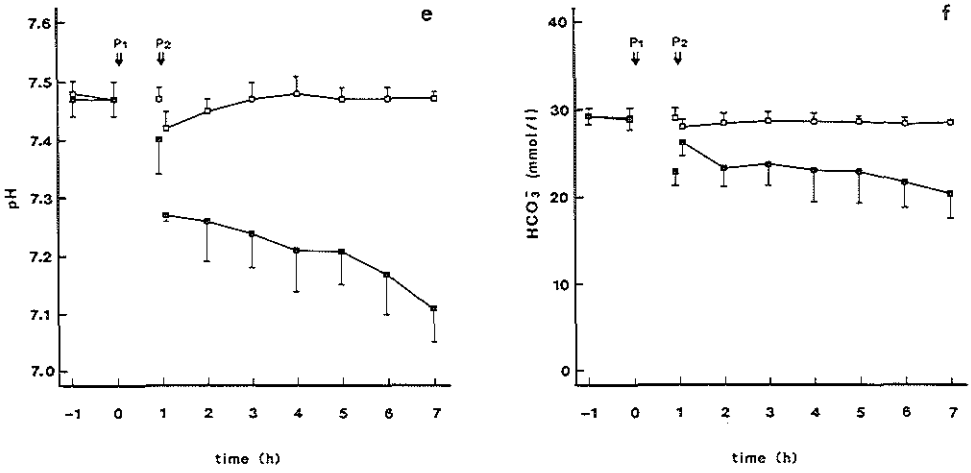

Fig. 3.2e and f.

mmHg ($p<0.001$) shortly after the first pair of lavages and recovered to different values in the first hour (Fig. 3.1c). After the second pair, P_{aO2} was on average 47.8 $\pm$ 11.9 mmHg ($p<0.001$) compared with baseline) and did not change significantly in all animals (Fig. 3.2a). In six animals this value was maintained for six hours. Two experiments ended at three and three and a half hours after the last lavage because of pneumothorax and technical problems respectively. In both experiments P_{aO2} was at the same level as in the other six.

In group IV (n=3) P_{aO2} fell from 304 $\pm$ 27.8 mmHg to 40.2 $\pm$12.7 mmHg shortly after the first pair of lavages. The second pair was done half an hour after the first pair. Immediately after the second pair, P_{aO2} was 34.2 $\pm$ 1.0 mmHg. However, in two animals P_{aO2} recovered to 74 mmHg and 84 mmHg at the end of the experiments, whereas a third animal recovered to a P_{aO2} of 249 mmHg (Fig. 3.1d)

P_{aO2} of group III fulfilled the definition of respiratory distress (30) and was satisfactorily stable for six hours (Fig. 3.2a). Therefore, we restricted the description of other variables to group III. The presented data include the data obtained from the two experiments that were studied for less than the whole six hour period. For statistical analyses however, we confined the group to the six experiments studied for all six hours.

In group III, S_{aO2} (Fig. 3.2b) decreased from 99.8 $\pm$ 0.3 % in baseline to

52.5 ± 20.8 % ($p<0.01$) immediately after the last pair of lavages. Venous admixture (Fig. 3.2c) increased from 5 ± 2 % to 50 ± 21 % ($p<0.01$). Both variables did not change significantly during the next six hours.

P_{aCO2} (Fig. 3.2d) was increased from 41.2 ± 2.9 mmHg in baseline to 63.8 ± 9.5 mmHg ($p<0.05$) immediately after the last lavage and V_D/V_T from 33 ± 4 to 56 ± 5 % ($p<0.001$), accompanied by a decrease in pH (Fig. 3.2e) from 7.46 ± 0.03 to 7.26 ± 0.07 ($p<0.001$), whereas HCO_3^- concentration (Fig. 3.2f) decreased from 29.0 ± 0.9 $mmol.l^{-1}$ to 23.2 ± 1.6 $mmol.l^{-1}$ ($p<0.001$). P_{aCO2} and V_D/V_T increased slightly until about 4 h after the last lavage (t=5 h). Then, a progressive rise in P_{aCO2} to 92.2 ± 11.2 mmHg and in V_D/V_T to 70 ± 3 % occurred (MANOVA, linear trend $p<0.01$ and quadratic trend $p<0.01$). pH decreased steadily to 7.11 ± 0.06 at the end; the steeper slope of the averaged values was not significant. HCO_3^- concentration did not change significantly.

Immediately after the last pair of lavages hemoglobin (Hb) concentration (Fig. 3.3) was 7.1 ± 0.8 $mmol.l^{-1}$, which was significantly higher than the baseline value of 5.9 ± 0.7 $mmol.l^{-1}$ ($p<0.001$). Hb concentration did not change significantly during the post-lavage period. It was significantly higher than that in the control group (analysis of covariance with the baseline values as covariates, $p<0.001$).

Pulmonary data

The tracheal pressure at peak insufflation ($P_{T,p}$) increased sharply from 19 ± 3 to 30 ± 3 cmH_2O ($p<0.001$) after the first pair of lavages and to 36 ± 3 cmH_2O ($p<0.001$) immediately after the second pair, followed by a gradual increase to 47 ± 2 cmH_2O at the end of the experiments ($p<0.01$, Fig. 3.4).

V_{EE} decreased from 23.0 ± 2.5 $ml.kg^{-1}$ in baseline to 13.7 ± 2.1 $ml.kg^{-1}$ after the lavages (n=6, $p<0.001$) and C_{rs} from 1.7 ± 0.2 ml. $cmH_2O^{-1}.kg^{-1}$ to 0.6 ± 0.1 $ml.cmH_2O^{-1}.kg^{-1}$ (n=6, $p<0.001$). In Fig. 3.5 an individual example of the pressure volume relationship before and after lavages is presented. The values of V_{EE} and C_{rs} after lavages were also lower than those in the control group ($p<0.001$).

The weight of lungs and heart was not determined in all animals because of technical problems. The weight of lungs and heart normalized to body weight of the lavage group, 27.2 ± 2.2 $g.kg^{-1}$ (n=4), was larger than that of the control animals, 20.7 ± 4.7 $g.kg^{-1}$ (n=6, $p<0.01$).

On the chest X-rays, generalized infiltrations and air bronchograms, both indicating alveolar edema, were observed after the lavages. These observations were confirmed by histologic examination of lung tissue after the lavages, which

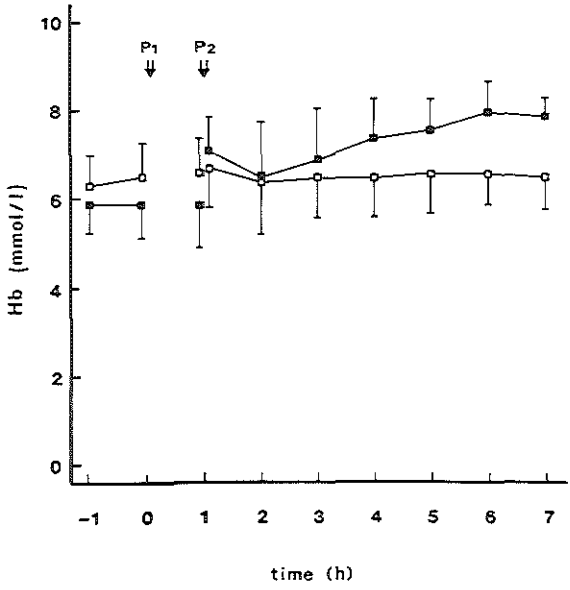

Fig. 3.3. Hemoglobin concentration.
Time scale, symbols and vertical bars as in Fig. 3.2.

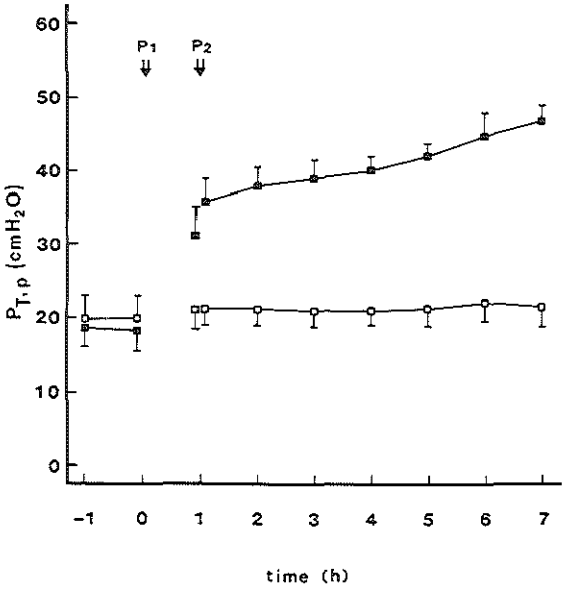

Fig. 3.4. Peak tracheal pressure.
Time scale, symbols and vertical bars as in Fig. 3.2.

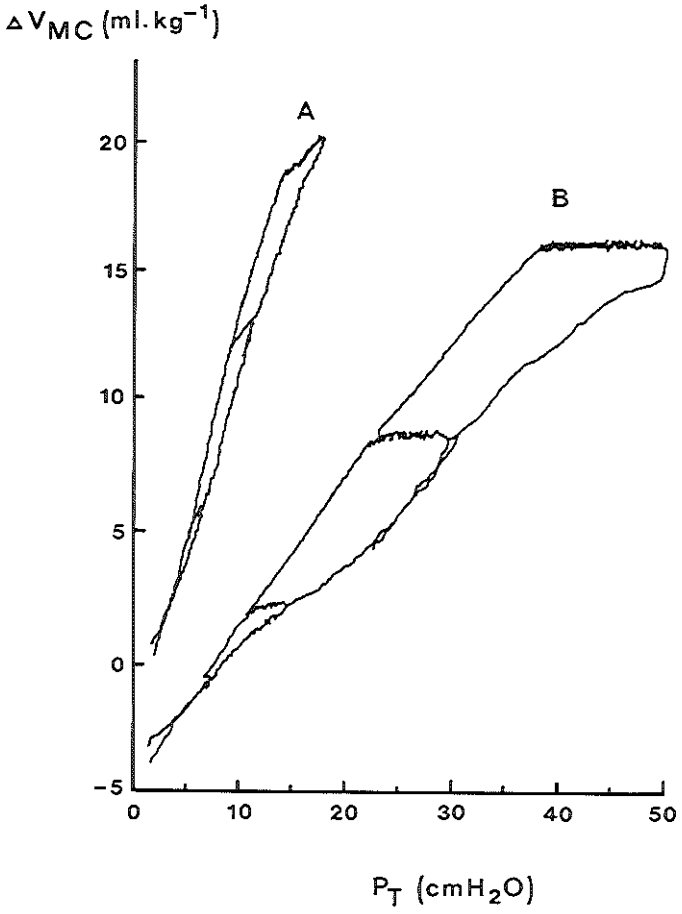

Fig. 3.5. Total respiratory compliance.
Change in thoracic volume (ΔV_{MC} versus airway pressure (P_T). A: baseline curve and B: curve after lavages. Zero thoracic volume on the Y-axis corresponds with end-expiratory lung volume in baseline condition.

revealed acute focal or diffuse bronchopneumonia in all lobes (Fig. 3.6), varying from mild to severe. It was sometimes associated with marked granulocytic infiltration of the interstitium. There was generalized severe interstitial edema of interlobular septa with pronounced dilation of lymphatics. These alterations showed no significant preference for any of the lobes. In one animal there was mild vasculitis. In another animal a single recent thrombus was found. Hyaline

membranes were not observed.

After lavage, the electron microscopic examination of the pneumocytes II (Fig. 3.7) showed a pronounced reduction in number of lamellar bodies as compared to the control group.

Hemodynamic variables

The baseline value of cardiac output (Q'_t) was on average 2.1 $\pm$ 0.2 $ml.s^{-1}.kg^{-1}$ and did not change significantly after the second pair of lavages: 2.4 $\pm$ 0.7 $ml.s^{-1}.kg^{-1}$ (Fig. 3.8a). Mean Q'_t did not change significantly during the post-lavage period, which was also true for the majority of individual experiments. In two experiments, however, Q'_t was unstable for the first 2 h of the post-lavage period. After lavages Q'_t was higher than in the control group (MANOVA with the baseline value as covariate, $p=0.02$). At the end of the experiments Q'_t was 2.2 $\pm$ 0.5 $ml.s^{-1}.kg^{-1}$in the lavage group and 1.7 $\pm$ 0.2 $ml.s^{-1}.kg^{-1}$ in the control group.

Aortic pressure (P_{ao}) was 95.5 $\pm$ 14.4 mmHg under baseline conditions and did not change significantly after the second pair of lavages until the last hour (Fig. 3.8b). Then, P_{ao} decreased to 64.2 $\pm$ 17.4 mmHg ($p<0.05$).

Pulmonary arterial pressure (P_{pa}) increased from 11.6 $\pm$ 3.6 mmHg to 29.6 $\pm$ 6.6 mmHg ($p<0.001$) shortly after the last pair of lavages and did not change significantly during the next 5 hours (Fig. 3.8c). After this period P_{pa} decreased slightly to 27.3 $\pm$ 5.7 mmHg ($p<0.01$).

Central venous pressure (P_{cv}, Fig. 3.8d) and pulmonary capillary wedge pressure (P_{pcw}) did not change significantly throughout the experiments in both the lavage and the control group.

Oxygen delivery (D_{O2}) decreased from 30.1 $\pm$ 5.2 $ml.s^{-1}.kg^{-1}$ to 21.4 $\pm$ 4.4 $ml.s^{-1}.kg^{-1}$ ($p<0.05$) immediately after the second pair of lavages and did not change significantly until the last hour (Fig. 3.9), when D_{O2} decreased to 18.7 $\pm$ 6.6 $ml.s^{-1}.kg^{-1}$ ($p<0.01$). In the post-lavage period D_{O2} was significantly smaller than in the control group (MANOVA constant trend between groups, $p<0.05$).

Fluid balance

The fluid balance is presented in Table 3.2. The non-recovered fluid from four lavages was 18.4 $\pm$ 2.7 % in total. The lavage fluid not recovered after the first pair of lavages was larger than that after the second pair (14.5 $\pm$ 1.9 ml kg^{-1}) and 11.1 $\pm$ 2.5 $ml.kg^{-1}$ respectively, $p<0.05$). The total fluid accumulation

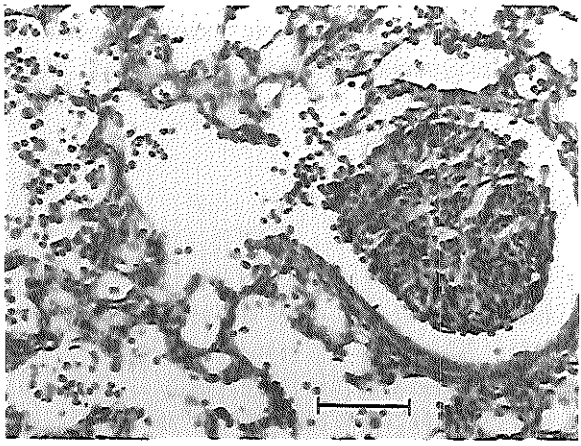

Fig. 3.6. Histology of the lungs after lavage.
Acute bronchopneumonia in pig lung, following lavage. Granulocytes are present in a bronchiole and in alveolar spaces (Hematoxylin and eosin; x150). Bar, 100 μm.

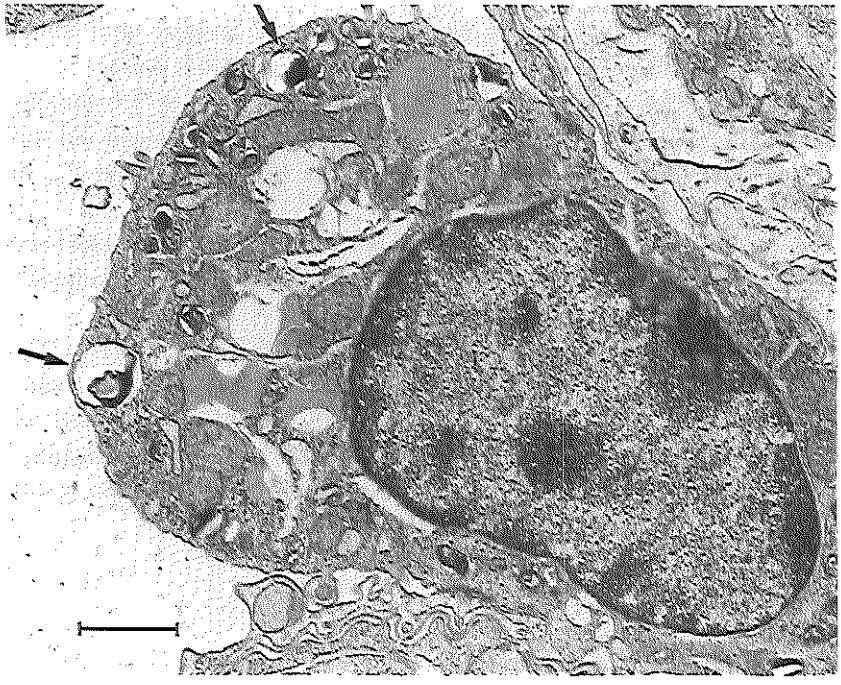

Fig. 3.7. Electron micrograph of pneumocyte type II in pig lung following lavage.
Lamellar bodies (arrows) are reduced in number and show signs of degeneration (x7000). Bar, 1 μm.

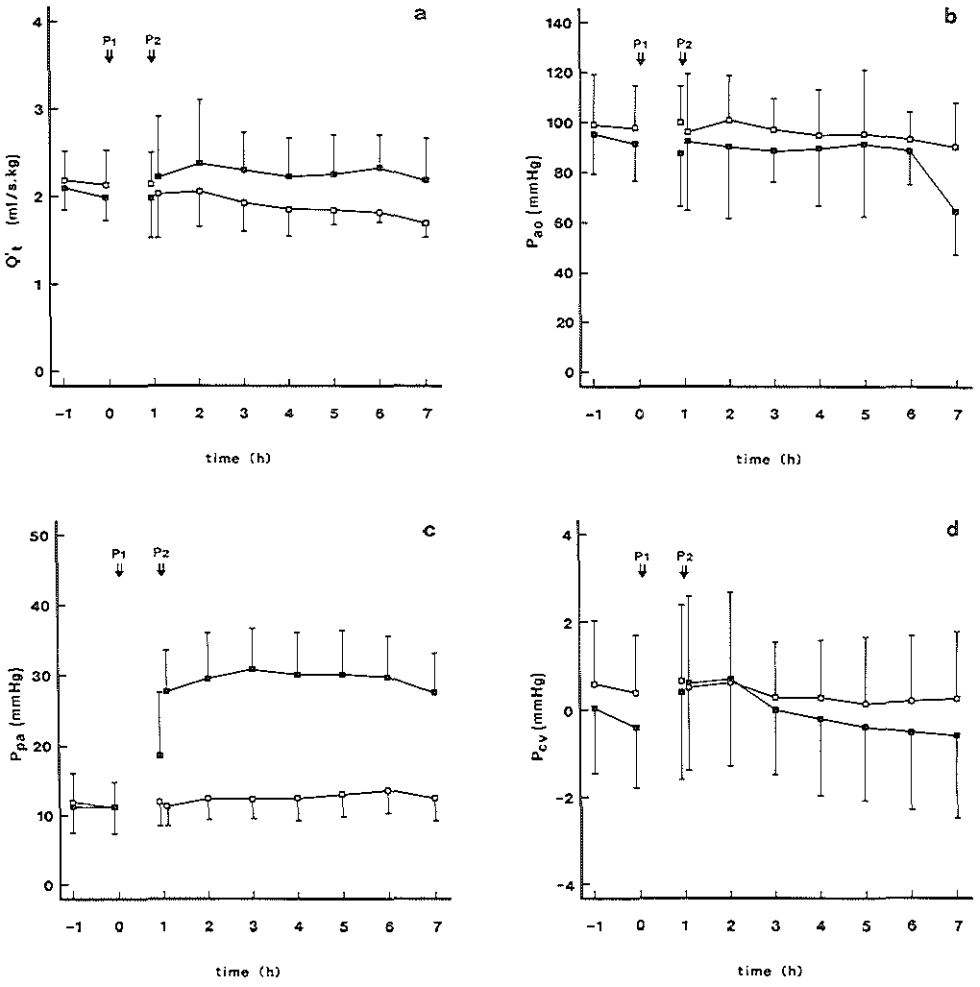

Fig. 3.8. Hemodynamic data.
Time scale, symbols and vertical bars as Fig. 3.2. a. Cardiac output, b. aortic pressure, c. pulmonary arterial pressure, d. central venous pressure.

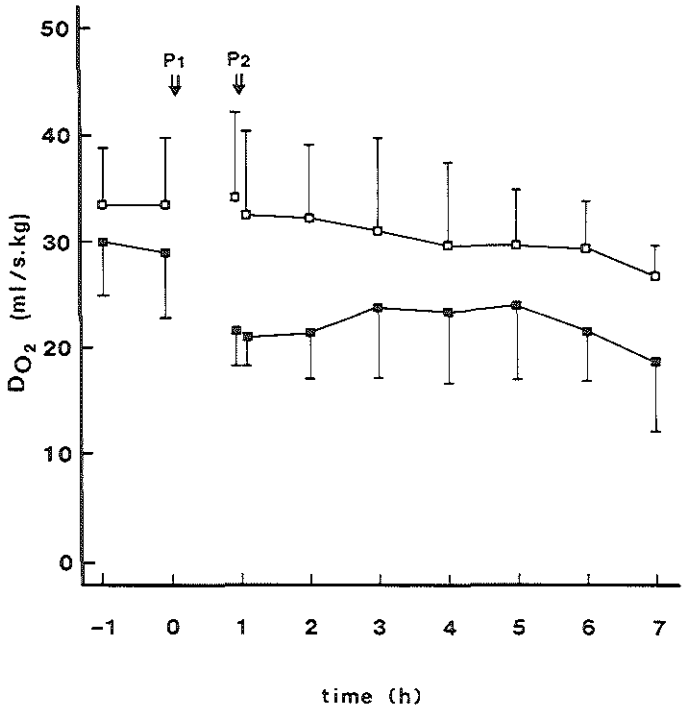

Fig. 3.9. Oxygen delivery.
Time scale, symbols and vertical bars as Fig. 3.2.

in the lavage group was almost twice that in the control group.

Criteria of respiratory distress

The lung injury score according to Murray (29), obtained by dividing the aggregate sum of the individual values by the number of components was 2.5. The chest roentgenogram showed alveolar edema in all lung lobes (value =4), the hypoxemia score (P_{aO2}/F_{IO2}) was below 100 (value =4) with a positive end-expiratory pressure of below 5 cm H_2O (value =0). The score of total respiratory compliance in our pigs was 2, being in the range of 0.57 to 0.84 ml.cmH_2O^{-1}. kg^{-1}.

Table 3.2. Fluid balance.

	Lavage mean ($ml.kg^{-1}$)	sd	**Control** mean ($ml.kg^{-1}$)	sd
Infusions	34.2	2.2	33.3	2.4
Non-recovered lavage fluid	25.7*	3.3	-	
Water production by metabolism	2.1		2.1	
Input:	62.0		35.4	
Urine production	18.6*	10.9	6.8	2.6
Evaporation				
via ventilation	3.5		3.5	
via skin	2.9		2.9	
Output:	25.0		13.2	
Total fluid balance	37.0		22.2	

* significant difference between lavage and controle group $p<0.05$. Water production by metabolism was estimated from the following determined variables: oxygen uptake was on average about 3.5 $l.kg^{-1}$ over the whole experiment and R.Q. about 0.8. Water production by carbohydrates and fats is about 0.80 and 0.48 ml per l oxygen consumption respectively (9). At an RQ of 0.8 oxygen is divided over carbohydrates and fats according to 1:2, giving 0.27 and 0.32 $ml.kg^{-1}$ water production per liter oxygen respectively, which is in total 0.59 ml water per l oxygen. Fluid loss via ventilation was estimated from the averaged ventilation close to 2 l. min^{-1}, inspiratory air of a dry oxygen-nitrogen mixture of 22 °C and expiratory air of 38 °C with fully saturated water vapour. Water loss via the skin by evaporative heat of water (0.58l $kCal.g^{-1}$). Heat loss by evaporation via the skin was assumed to be 10% of total heat production (28). Heat production was calculated from oxygen uptake and caloric heat of oxygen, i.e. 4.8 kCal at R.Q. = 0.8.

DISCUSSION

Control experiments

Sham lavages were performed with an apnea of 90 s at end-expiratory lung volume. An inspiratory volume of 35 ml per kg body weight would have resulted in the same amount of lung stretch as during the lavages with 35 ml saline per kg. However, we aimed at a simulation of the same amount of oxygen in the lungs during the apneic periods. P_{aO2} was slightly but significantly decreased by the sham lavages. This decrease of 20 mmHg did not change gas transport, because at a level of about 300 mmHg blood is fully saturated.

We did not find any immediate effect of the sham lavages on cardiac output. Therefore, we regarded the gradual decrease in cardiac output during the post sham lavage period due to other experimental conditions (Fig. 3.8a). We have no data to explain the decreased total respiratory compliance in the sham lavages group.

The overall fluid balance was 22.2 $ml.kg^{-1}$ positive in the control group, whereas Hb concentration remained constant. Probably this fluid accumulated in the peripheral tissues during the experiments, increasing body weight by only 2 %.

The lavage regimes

In the series of single lavages a profound fall in P_{aO2} to about 50 mmHg was found after the second lavage. Two lavages at an interval of 5 min caused approximately a similar profound fall in P_{aO2} (Figs.1a and 1b). In both series a partial recovery followed which was smaller after the second single lavage than after a pair of lavages. Recovery was different in time course in the different experiments of both series. A third single lavage decreased P_{aO2} to a value of about 50 mmHg, which remained stable for several hours. This was also found after the second pair of lavages in the second series in spite of the larger previous recovery. Thus, to wash out enough surfactant to establish a stable respiratory distress two pairs of lavages at an interval of 3-5 hours were as effective as 3 single lavages in a period of 5-6 hours. Our attempt to shorten the interval between the two pairs of lavages succeeded in the third series with an interval of one hour (Fig. 3.1c), but failed in the fourth series when the interval was shortened to half an hour (Fig. 3.1d). We concluded

that a critical time interval between 30 and 60 minutes after a first pair of lavages had to be surpassed to obtain a stable distress after a second pair.

For several reasons we have not tried to specify the critical time interval more accurately. First, we assumed the critical time interval between the first and second pair of lavages not to be similar in all animals, because of differences in recovery after the second pair of lavages in the group with an interval of 30 min. Therefore, we considered an interval of 45 min too much a hazard to get a stable respiratory distress. Second, we did not want to sacrifice more animals for a trivial gain of time compared with the total duration of the experiments. Third, a series of repetitive lavages at five minutes intervals to obtain a distress, lasted also about one hour (26,36).

Our data indicate that a regime of 6-13 repetitive lavages at intervals of 5 minutes is a superfluous regime. It cannot be excluded that more apneic periods than the four in our study caused extra deleterious effects. Moreover, such an extensive regime might have caused a much larger fluid load. These effects were not evaluated in those studies (10,13,16,24,26,32,36).

Surfactant release and recovery of gas exchange

Surfactant release from the stores in the pneumocytes II (3,11) needs time (21). A minimal time interval between the two pairs of lavages had to be surpassed to obtain a stable respiratory distress without recovery. We suppose that this minimal interval between 30 and 60 minutes was necessary to release the stores maximally and that the second pair of lavages washed out this released surfactant. Extensive surfactant release was confirmed by the electron microscopic images of the pneumocytes II (Fig. 3.7), which showed an almost complete depletion of the lamellar bodies. Obviously the post-lavage period of six hours was too short to recover the surfactant stores of the pneumocytes II in pigs.

We additionally hypothesized that the time course of recovery after the first pair of lavages (Fig. 3.1b and 3.1c) was greater than that of release of surfactant. We assumed a depletion of the stores of the pneumocytes II after 60 min whereas recovery was not yet completed. A delayed recovery with respect to surfactant release could be due to the time necessary for resorption of the non-recovered lavage fluid and the time to spread out surfactant on the alveolar lining. Qualls et al. (31) reported a mean half life time of 15.5 $\pm$ 0.8 min for 6 $ml.kg^{-1}$ saline instilled into the airways of dogs. If the instilled volume disappeared exponentially from the alveoli, the amount left after one hour was about 6 % (= 0.5^4 x 100 %). In our experiments the non-recovered

lavage fluid was on average 14.5 ± 1.9 ml.kg^{-1} after the first pair of lavages. If half life time is the same for pigs and for a two and a half times larger volume as studied by Qualls et al., about 0.9 ml. kg^{-1} of the lavage fluid was left in the lungs at the moment of the second pair of lavages. The difference in non-recovered volume between the first and the second pair of lavages was 3.5 ml.kg^{-1}, which is 24 % of the residual alveolar fluid after the first pair. If this difference is an indication of the amount of residual fluid after one hour, it is more than the 6% in Qualls' results. This might depend on the initial amount of residual alveolar fluid. We do not know how much P_{aO2} was decreased by an amount of 3.5 ml.kg^{-1} alveolar fluid. We assume that this fluid was part of areas with atelectasis, contributing to the delayed recovery.

The distress model

Functional changes

Gas exchange

According to the expanded definition of respiratory distress by Murray (29) our pigs had moderate to severe respiratory distress.

P_{aO2}, as a main indicator of respiratory distress was maintained in all series with a stable distress at a level close to 50 mmHg. This level is the same as the main criterion of ARDS at an F_{IO2} of 0.6 (30). We cannot satisfactorily explain why all distress regimes have stabilized at a P_{aO2} level close to 50 mmHg. Perhaps this level has the importance of a threshold value, i.e. the lowest level to maintain sufficient oxygen extraction. Presumably, counteracting control mechanisms of circulation and gas exchange prevent a further decrease. We ascribed the decrease in P_{aO2} and in S_{aO2} and the increase in venous admixture to lung areas with low ventilation-perfusion ratios. We also assumed areas with increased ventilation-perfusion ratios to explain the increased P_{aCO2} and physiological dead space. pH decreased in accordance with the increase in P_{aCO2}, because bicarbonate concentration remained approximately constant. In contrast to the steeper rise in P_{aCO2} during the last two hours the fall in pH was not significantly different from the fall in the early post-lavage period. This could be a result of random noise.

Pulmonary variables

Assuming a constant chest wall compliance, changes in total respiratory compliance indicated changes in lung compliance. Total respiratory compliance was lowered, which is in agreement with literature data

(24,26,33). This decrease could be due to an increase of surface tension in the alveoli because of loss of surfactant (12) and to a decrease in end-expiratory lung volume. The decrease in compliance at corresponding thoracic volume, i.e. at the same stretch of lung tissue, indicated an increased stiffness of the lungs after the lavages.

Gattinoni et al. (15) pointed out that in patients with acute respiratory failure the P-V curves of the lungs are mainly dependent on the residual healthy parts of the pulmonary tissue. The diseased parts should not primarily contribute to the slope of the P-V curve, giving a normal compliance per liter of lung volume in ARDS patients. Our findings in animals are not in agreement with these results. A reason could be a more homogeneously distributed decrease in surfactant after extensive lung lavages.

Circulation

Studies on functional changes in the circulation after lavages are scarce. We did not find any data under conditions of constant ventilatory parameters. In some papers (13,22,36) the effects of positive end-expiratory pressure and high frequency jet ventilation on cardiac output have been presented.

In our experiments the averaged data of cardiac output and aortic pressure were stable and not different from their baseline values during five hours after the last pair of lavages (t=6 h). Two individual experiments showed some instability during the first two hours of the post lavage period. Thereafter, cardiac output and aortic pressure remained also stable. Cardiac output was maintained at a significantly higher level in the lavage group than in the control group, which we ascribed to autonomic stimulation of the cardiovascular system in response to the low P_{aO2} and the high P_{aCO2} (25). In the last hour of the post-lavage period arterial pressure decreased whereas cardiac output did not change. This indicates a fall in resistance (R_s) of the systemic circulation. We have not presented the data of R_s because it showed the same pattern as P_{ao}. We assume this fall in R_s to be the result of prolonged hypoxia and the development of acidosis in the peripheral tissues (25).

Pulmonary arterial pressure (P_{pa}) was increased after each pair of lavages. In the post-lavage period the high P_{pa} was maintained. We assume this rise due to pulmonary vasoconstriction by hypoxia and vasoactive metabolites from granulocytes (10).

Heart function

We had no indication that heart function was deteriorated by lavages, since cardiac output, aortic pressure, central venous pressure and pulmonary

wedge pressure were not changed. A rise in P_{pa} does not necessarily lead to a decrease in cardiac output (27), as we also found in our lavage experiments. Increased sympathic tone may also have contributed in maintaining heart function (25).

Oxygen delivery

In patients with respiratory failure an important variable to be controlled is oxygen delivery to the peripheral tissues (6,37). Compared to the control group, oxygen delivery was decreased during the post-lavage period. However, oxygen delivery remained sufficient after the lavages as was also indicated by a normal SBE.

Fluid balance

The weight of lungs and heart was on average 6.5 $g.kg^{-1}$ larger in the lavaged pigs than in the controls. Thus, a main part of all non-recovered lavage fluid (25.7 $ml.kg^{-1}$) was resorbed. The extra lung (and heart) weight indicated either the presence of a residue of lavage fluid in the alveoli, or an almost full resorption followed by an edema formation in the lungs throughout the post-lavage period. The resorbed lavage fluid and the infusion fluid did not accumulate in the blood, because in the post lavage period hemoglobin concentration was increased with respect to baseline. Total fluid load in the lavage pigs was 62 $ml.kg^{-1}$, which was partly compensated for by excretion of urine (18.6 $ml.kg^{-1}$) and fluid loss by evaporation (6.4 $ml.kg^{-1}$, Table 3.2). As 6.5 $ml.kg^{-1}$ of the net positive load of 37 $ml.kg^{-1}$ was found in lungs and heart, the lavaged animals accumulated about 30 $ml.kg^{-1}$ fluid, which is about 3 % of body weight. This is 1 % more than that in the control animals. We do not expect a large influence of this amount on the animals' condition.

Morphologic changes

Histologic examination of lung tissue after the experiments confirmed the pattern of edema on the X-ray observations at the end of the lavage period. Lung edema is probably caused by an influx of granulocytes into the lungs (Fig. 3.6) producing toxic substances (34,35) and by a decrease of surfactant after lung lavages (1,8). Hyaline membranes as reported by others in guinea pigs (26) and rabbits (16,33) were not observed, possibly because the time for their development was too short.

Conclusions

As an optimal regime for induction of respiratory distress in pigs we recommend a regime of two lavages within five minutes, followed after one hour by another pair of lavages.

This experimental model of respiratory distress is characterized by:
-a stable P_{aO2} of about 50 mmHg during at least six hours;
-a stable cardiac output, aortic pressure and central venous pressure during five hours with values similar to baseline values;
-a decreased, but sufficient oxygen delivery during five hours;
-a pulmonary hypertension for six hours;
-a decreased total respiratory compliance and a decreased end-expiratory lung volume;
-electron microscopic evidence of extensive depletion of the surfactant stores in the pneumocytes II;
-radiographic and morphologic features of lung edema and inflammation.

This animal model is satisfactory stable for many hours to perform studies on basic mechanisms and therapeutic interventions.

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CHAPTER IV

PULMONARY ARTERIES IN A LAVAGE MODEL OF RESPIRATORY DISTRESS
A MORPHOMETRIC STUDY IN PIGS

In patients with the adult respiratory distress syndrome (ARDS) pulmonary hypertension is a common feature (23). In the early phase of the disease, active vasoconstriction is likely to be the main cause of this increase in mean pulmonary arterial pressure. In a later phase pulmonary hypertension predominantly depends on structural alterations of the vessels (18,22).

Lung lavage with saline can be used as a model of respiratory distress in animals by washing out surfactant (13). The pulmonary vascular pressure profile after lung lavage indicates mainly an increase in pressure in the arteries, whereas the pressure drop across the venous segment remains small (16). The increase in mean pulmonary arterial pressure after lavage is at least partly caused by vasoconstriction under influence of products from neutrophylic granulocytes (7).

In the present study severity and distribution of active vasoconstriction in lavage induced respiratory distress were investigated. To determine severity the medial thickness of muscular pulmonary arteries in pigs was measured. To assess the distribution throughout the lungs, various parts were studied.

METHODS

Surgical procedures and ventilatory conditions

Twelve female Yorkshire pigs (5-7 weeks old, 9.4 $\pm$ 0.7 kg) were anesthetized with sodium pentobarbital (30 $mg.kg^{-1}$, intra-peritoneal) and placed in supine position. Body temperature was maintained at 38°C. After tracheostomy, the pigs were connected to a volume-controlled ventilator. A polythene single lumen catheter was inserted through the right common carotid artery into the aortic arch for measuring arterial blood pressure and sampling of blood. Three catheters were inserted via the right external jugular vein: a Swan-Ganz catheter (5F, American Edwards laboratories, Irvine CA, U.S.A.) into the left pulmonary artery for monitoring pulmonary arterial pressure and cardiac output and for sampling of mixed

venous blood, a double-walled catheter into the right atrium for injection of saline at room temperature to determine cardiac output with the thermodilution method, and a four lumen catheter into the vena cava for measuring central venous pressure and for infusion of fluids and anesthetics. Each blood pressure catheter was continuously flushed with 3 $ml.h^{-1}$ saline containing 10 IU heparine per ml to avoid obstruction by thrombi. A catheter was put into the urinary bladder to avoid retention of urine.

During the experiments, anesthesia was maintained by a continuous infusion of sodium pentobarbital (8.5 $mg.kg^{-1}.h^{-1}$). Tubocurarine (0.2 $mg.kg^{-1}$) was given to suppress spontaneous breathing.

After surgery the animals were connected to a computer controlled ventilator (11). At the start of the experiments tidal volume (V_t) was adjusted to an arterial carbon dioxide tension (P_{aCO2}) of about 40 mmHg. Ventilatory settings were: a frequency of 10 breaths per minute, an inspiratory fraction of oxygen of 0.6, a positive end-expiratory pressure of 2 cmH_2O, a ratio of inspiration: expiration of 2:3. These parameters were not changed throughout the experiments.

Measurements

Gas exchange

Oxygen and carbon dioxide tensions and the acid-base indices were determined with an automatic blood gas analyzer (Radiometer ABL3, Copenhagen, Denmark), and hemoglobin (Hb) and oxygen saturation (S_{O2}) with use of an oxymeter (Radiometer OSM2, Copenhagen, Denmark). Inspiratory and mixed expiratory gases were analyzed by a mass spectrometer (Perkin-Elmer, MGA 1100, Pomona, California U.S.A.). Venous admixture (Q'_s/Q'_t) was calculated in percentage of cardiac output according to:

$$Q'_s/Q'_t = 100 \times (C_{cO2}-C_{aO2})/(C_{cO2}-C_{\bar{v}O2})$$

Oxygen content in arterial (C_{aO2}), mixed venous ($C_{\bar{v}O2}$) and pulmonary end-capillary blood (C_{cO2}) were calculated according to:

$$C_{xO2} = (0.0031P_{xO2}) + (S_{xO2} \times 1.39Hb)/100$$

where subscript x stands for arterial, mixed venous and end-capillary blood respectively. In this equation, 0.0031 is the solubility of oxygen in blood in ml O_2 per 100 ml and per mmHg and 1.39 is the oxygen binding capacity in ml O_2 per g Hb. The saturation of the pulmonary end-capillary blood was derived from the oxygen saturation curve of pig blood and the ideal alveolar oxygen tension (P_{AO2}) as a substitute of the pulmonary end-capillary oxygen tension. The parameters in the equation of the human oxygen saturation curve were fitted for the pig's oxygen saturation curve, based on pig blood data from other experiments in our laboratory.

This oxygen saturation curve was in accordance with the curve described by Bartels and Harms (3). P_{AO2} was derived from Anthonissen and Fleetham (2).

Physiological dead space (V_D) in percentage of V_T was obtained from the equation:

$$V_D/V_T = 100 \times (P_{aCO2} - P_{\bar{E}CO2}) / P_{aCO2}$$

where $P_{\bar{E}CO2}$ is the mixed expiratory carbon dioxide tension.

Circulation

Blood pressures were measured relative to atmospheric pressure at manubrium level using Statham transducers (Gould, Hato Rey, Puerto Rico) and averaged over a ventilatory cycle. Transducers were calibrated by application of pressure to this reference level under guidance of a mercury manometer.

Cardiac output was determined by thermodilution. Four determinations equally spread over the ventilatory cycle were averaged and used as an estimate of mean cardiac output (10).

Experimental procedures

Lavages

After the surgical procedures and a stabilisation period baseline measurements were obtained during one hour. In the lavage group one pair of lung lavages each with 35 $ml.kg^{-1}$ saline at an interval of 5 min was done, followed after one hour (animals No.7 to 10) or after half an hour (animals No.11 and 12) by another pair of lavages. After the lavages these animals were observed for about six hours for physiological observations, implying a total period of mechanical ventilation of at least eight hours. In the control group sham lavages were performed by interruptions of ventilation at end-expiration for 90 sec being the time needed for a lavage. These animals were mechanically ventilated for the same time as the animals in the lavage group.

The presented variables of gas exchange and hemodynamics were determined at the end of the experiments shortly before the animals were killed.

Morphology and morphometry

At the end of the experiments, the animals were killed with 70 $mg.kg^{-1}$ body weight pentobarbital. One animal (No.12, Table 4.2) died spontaneously at about 6 h after the last lavage. The lungs were fixed immediately after death by instillation of formalin through the trachea at 30 cmH_2O pressure. After ligation of the blood vessels, heart and lungs were removed from the body and weighed. In order to exclude possible effects of hydrostatic

pressure, blocks were taken from the ventral part of the upper and the dorsal part of the lower lobes from either side. Histologic slides, cut from these blocks and stained with hematoxylin and eosin and with an elastic-van Gieson stain, were investigated. The slides stained by the elastic stain were used for measuring pulmonary arterial medial thickness. All measurements were carried out by one of the authors (N.W.). Only muscular arteries with a circular or near circular cross-section were measured. Medial thickness, being the distance from internal to external elastic lamina, was expressed as a percentage of external diameter (21). Data were obtained from 25 arteries for each lobe, a total of 100 per animal.

Electron microscopy

Blocks for electron microscopy were taken from the middle lobe of the lungs. These blocks were immediately fixed in glutaraldehyde. The blocks were post-fixed in 1 % osmium tetroxide at 4°C, dehydrated in acetone and embedded in LX112 (epoxy resin, Ladd research industries, Burlington, Vermont, U.S.A.). Ultrathin sections (LKB ultratome IV) were mounted on copper grids (200 mesh) and contrasted with uranyl acetate (10 min at 45°C) and lead citrate. They were examined with a Zeiss 902 electron microscope (Oberkochen, Germany).

statistical analysis

Results were analyzed using Wilcoxon's tests for paired and unpaired samples. $p \leq 0.05$ was accepted as statistically significant. Data are presented as mean $\pm$ 1 sd.

RESULTS

Gas exchange, hemodynamic variables and lung weight

Variables of gas exchange and circulation at the end of the experiments are presented in Table 4.1. The main features of the animals were an arterial hypoxemia and hypercapnia, an increase in venous admixture and a pulmonary hypertension. In the two animals (No.11 and 12) with the pairs of lavages at half an hour interval P_{aO2} (74 and 84 mmHg respectively) was somewhat higher than in the other four animals (No.7 to 10). However, P_{pa} was similar and stable for all animals. The weight of lungs and heart in the

Table 4.1. Gas exchange and hemodynamic variables at the end of the experiments in the control group and the lavage group.

	control		lavage	
P_{aO2} (mmHg)	284	± 21	53	± 13**
$P_{\bar{v}O2}$ (mmHg)	44	± 3	31	± 7*
P_{AO2} (mmHg)	392	± 21	331	± 19*
S_{aO2} (%)	100	± 0	55	± 18**
P_{aCO2} (mmHg)	39	± 2	84	± 11**
pH	7.47	± 0.01	7.14	± 0.07**
HCO_3^- (mmol.l^{-1})	27.8	± 0.7	26.1	± 1.6*
V_D/V_T (%)	33	± 4	66	± 3**
Q'_s/Q'_t (%)	4	± 1	58	± 14**
Q'_t (ml.s^{-1}.kg^{-1})	1.8	± 0.1	2.0	± 0.4
P_{ao} (mmHg)	93	± 11	88	± 18
P_{pa} (mmHg)	14	± 4	31	± 6**
P_{cv} (mmHg)	0.1	± 1.4	0.7	± 1.9

Mean ± sd; *: $p<0.01$ and **: $p<0.001$ compared to controls.

lavage group was 29 ± 3 g per kg body weight, whereas that in the controls was 21 ± 5 g per kg body weight ($p<0.01$).

Morphology

Histologic examination of lung tissue from the experimental animals after the lavages revealed acute focal or diffuse bronchopneumonia in all lobes. Sometimes there was also marked infiltration of the interstitium by granulocytes. The interlobular septa showed prominent broadening by severe generalized interstitial edema with pronounced dilation of lymphatics. These alterations showed no significant preference for any of the lobes. In one animal there was mild vasculitis in an artery. In another animal a single recent thrombus in an artery was found. There were no hyaline membranes. Most muscular pulmonary arteries showed a medial thickness far greater than in the control animals. Constricted muscular arteries appeared to be clustered. There was a prominent crenation of internal and external elastic laminae (Fig. 4.1), while the endothelial cells protruded into the lumen.

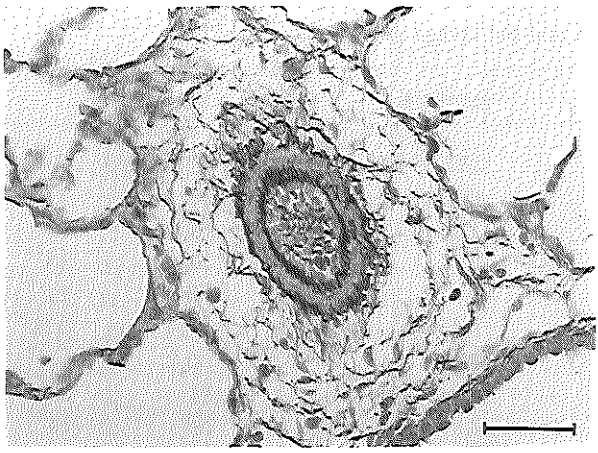

Fig. 4.1. Muscular pulmonary artery after lavage.
Medial thickness was increased. There was prominent crenation of the internal and external elastic laminae. (Elastic-van Gieson stain, x 150). Bar, 100 μm.

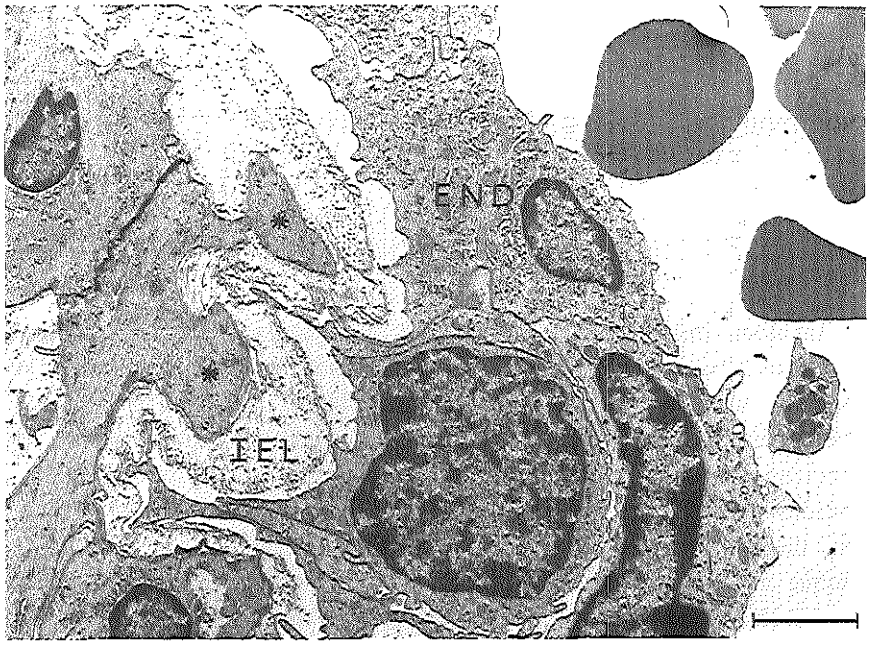

Fig. 4.2. Electron micrograph of muscular pulmonary artery.
The endothelial cells (END) appear swollen and protrude into the lumen. The internal elastic lamina (IEL) is prominently crenated with herniations of smooth muscle cell cytoplasm (*) between the folds (x3000). Bar, 2.3 μm.

The exceedingly thin walls of pulmonary veins make morphometric approach difficult. In the lavage animals, however, it was suggestive that there was regularly mild thickening of the walls of pulmonary veins and venules.

Electron microscopy

In muscular pulmonary arteries endothelial cells were swollen and protruded into the lumen. There were occasionally vacuoles in the cytoplasm. The muscular arteries showed constriction with pronounced crenation of the internal elastic lamina and with herniations of the cytoplasm of the smooth muscle cells (Fig. 4.2).

Morphometry of pulmonary arteries

Examples of the distribution of medial thickness in the left upper lobe of a control animal and of an animal that received lavages are shown in histograms (Fig. 4.3a and 4.3b).

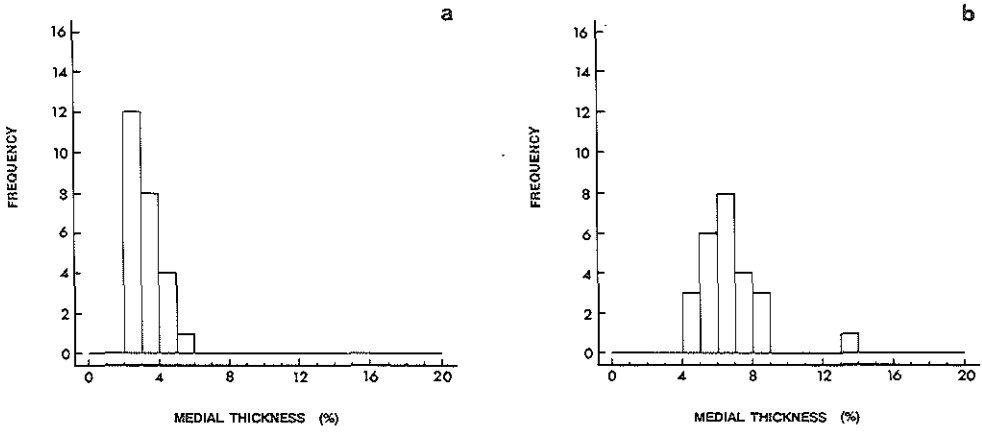

Fig. 4.3. Frequency distribution of medial thickness.
Left upper lobe of a) control and b) animal after lavage. Medial thickness, as estimate of vasoconstriction, was determined for 25 muscular pulmonary arteries per lobe.

The overall mean value of medial thickness was 3.7 ± 1.5 % in the control group and 7.7 ± 2.9 % in the lavage group ($p<0.01$). The animals of the lavage group had a greater medial thickness (Table 4.2) than the controls in left upper ($p<0.01$), right upper ($p<0.01$) and right lower lobes ($p<0.01$). The value for the left lower lobe in the lavage group was not significantly different from that in the control group ($p=0.09$). There was no significant difference in any of the groups between upper ventral and lower dorsal lobes. In the control group medial thickness was relatively great in the right upper lobe of three animals and in the left lower lobe of two others.

The standard deviation of medial thickness in each lobe was larger in the lavage group than in the control group ($p<0.01$, Table 4.2).

Table 4.2. Medial thickness

	LU	LL	RU	RL
control group				
1	3.3(0.9)	2.4(0.3)	5.2(2.0)	3.4(0.8)
2	3.5(0.9)	3.5(0.5)	5.2(1.2)	3.5(0.7)
3	3.3(0.8)	3.2(0.6)	5.5(1.3)	3.1(0.7)
4	3.8(0.8)	3.3(0.9)	3.9(1.7)	3.1(0.6)
5	3.5(0.8)	8.5(2.3)	3.4(0.7)	3.8(0.8)
6	3.3(0.5)	7.2(2.1)	4.6(2.0)	4.0(0.8)
lavage group				
7	5.1(2.0)	8.2(2.4)	6.3(2.4)	5.8(1.2)
8	8.6(4.1)	6.8(2.6)	10.9(3.7)	5.6(1.6)
9	11.7(4.1)	7.3(1.8)	8.2(2.3)	7.4(2.4)
10	7.5(2.1)	6.7(1.8)	8.3(2.7)	6.3(1.2)
11	7.6(5.4)	9.0(2.9)	5.9(2.8)	9.0(5.5)
12*	7.3(3.3)	8.4(5.1)	8.1(3.6)	8.0(3.2)

Medial thickness (% of external diameter) ± (sd) for the individual animals of the control group and the lavage group. Each row represents the data of medial thickness from one animal. * animal that died spontaneously. LU and RU: left and right upper lobes, LL and RL: left and right lower lobes.

DISCUSSION

The lavage model

Lung lavages were used to wash out surfactant and to induce respiratory distress in animals (13). Without sufficient surfactant the alveoli tend to collapse. Because in the present study the lungs were fixed immediately after death by instillation of formalin through the trachea at 30 cmH_2O pressure, we assumed that collapsed alveoli opened again. This could explain why only a slight focal atelectasis was observed with the light-microscopic examination, whereas others found a widely distributed focal atelectasis (13,15).

Histologic investigation showed severe interstitial edema and dilation of lymph vessels following lavage. This is consistent with the increased lung weight. The pulmonary edema following lavages, also found by others (7,9,12), is probably caused by products of neutrophylic granulocytes (12,17,19) and by lack of surfactant (1,6). A widespread obstruction by thrombi as seen in the adult respiratory distress syndrome (20), was not observed in our animals.

After lavage P_{aO2}, S_{aO2} and $P_{\bar{v}O2}$ were decreased as compared to the control group by an increase in venous admixture. The rise in P_{aCO2} was attributed to overall hypoventilation based on extensive ventilation-perfusion disturbances. Physiological dead space was increased. The rise in P_{aCO2} resulted in a severe respiratory acidosis as indicated by the very low pH and normal bicarbonate concentration. In the two animals with an interval of half an hour between the two pairs of lavages, pulmonary hypertension and respiratory distress were about the same as in the other four animals with one hour between the two pairs of lavages.

Vasoconstriction

In patients with ARDS, P_{aO2} is lowered and mean pulmonary arterial pressure is increased (23). After a long period of respiratory distress with mechanical ventilation, medial thickness is increased mainly due to medial hypertrophy (20). In ARDS patients endothelial cell swelling can reduce the vascular lumen at cross-section, whereas the capillaries can dilate in late stages of the disease (20). In the early stage of the disease pulmonary hypertension can be reversed by vasodilators, indicating that active vasoconstriction is an important factor (23).

In our study of acute respiratory distress, medial hypertrophy could not yet have been developed. Thus, we attributed the increase in medial thickness to

active vasoconstriction. This was also apparent from the pronounced crenation of the elastic laminae and the protrusion of the endothelial cells into the lumen (Fig. 4.2). The increase in mean pulmonary arterial pressure was a result of this active vasoconstriction.

Active constriction of pulmonary arteries in lavage induced respiratory distress may have been caused by two mechanisms: hypoxia and the influence of mediators from granulocytes that were numerous in lung tissue.

Local hypoxia in areas with atelectasis or edema after lavage could have resulted in local vasoconstriction.

Granulocytes, which were already present in recovered fluid from the last lavage (unpublished data), seem to be important in mediating lung injury following lavage (7,5,8). Granulocyte-depleted rabbits showed a preserved gas exchange and a lower protein leakage after lavage than animals with normal granulocytic function (12). It was shown that both indomethacin and thromboxane-A2 (TXA2) blocker could prevent a rise in mean pulmonary arterial blood pressure after lavage (7). P_{aO2}, however, remained low and protein leak was high after indomethacin and TXA2-blocker, indicating that pulmonary hypertension and edema were caused by different mediators.

Our data of vasoconstriction were probably not influenced by the pentobarbital anesthesia (4,22). Two animals from a pilot group and the one animal in the present study that died spontaneously had medial thicknesses comparable to the other lavage animals. This indicates that the lethal pentobarbital dose did not alter pulmonary vasoconstriction.

Vasoconstriction was not due to the apneic periods during the lavages, since in the control experiments the average medial thickness was not increased. In some control animals medial thickness was increased in one lobe, especially left lower and, to a lesser extent, right upper lobes. Presumably ventilation in these lobes was decreased. We have no data to verify this supposition.

Inhomogeneity of vasoconstriction

In patients with ARDS, lung lesions appear to be spread inhomogeneously (14). This is also true for the lung vessels, where the variance in medial thickness increases with duration of ARDS, indicating differences in medial hypertrophy (13).

Since the standard deviation of medial thickness was much greater in the lavage animals than in the controls, active vasoconstriction varies within each lung region. In the animals with a respiratory distress there was a scatter of the values of medial thickness. Normal vessels and clusters of more or less

constricted vessels were closely located within each region. The variation in vasoconstriction could have been due to unequal distribution of either atelectasis (13,15) or edema or both. The clustering of constriction in muscular arteries was probably the result of the fact that an artery contracts together with its branches.

Conclusions

In lavage induced respiratory distress there is severe vasoconstriction of muscular pulmonary arteries. The severity in vasoconstriction is not different between various parts of the lungs. Within each lung region there was a variation in medial thickness with normal vessels adjacent to clusters of constricted vessels. The vasoconstriction resulted in a rise in mean pulmonary arterial pressure. Vasoconstriction was attributed to local hypoxia and to mediators from granulocytes.

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CHAPTER V

EFFECTS OF PEEP IN EARLY RESPIRATORY DISTRESS IN PIGS BY LAVAGES

In patiënts with a respiratory distress syndrome (RDS), mechanical ventilation with a positive end-expiratory pressure (PEEP) is applied to improve gas exchange (2). PEEP, however, has negative effects on cardiac output (22,24,26,32,41). Several authors tried to define indicators for the appropriate level of PEEP, which was usually defined as the PEEP level where oxygen delivery to the tissues was maximal and which was called "best PEEP". Lung compliance was reported to be an indicator (34), which was not confirmed by others (13,24,26). The difference between arterial and end-tidal carbon dioxide tension was also proposed as an indicator (4,24), whereas other authors rejected this indicator (22). Venous admixture (14) and end-expiratory lung volume (11) were suggested. Others did not find any optimal conditions in PEEP studies (6,22,26,41). None of these authors compared all proposed indicators in the same subjects.

We studied the effects of stepwise increases in PEEP on gas exchange and hemodynamic variables in our model of early respiratory distress in pigs. We developed such a model of respiratory distress, which was stable for 6 h, by using a standardized regime of lung lavages (16). We correlated all variables mentioned above with oxygen delivery to evaluate whether they could be used as indicators of "best PEEP". We obtained strong evidence that none of these variables can serve as an indicator of "best PEEP" in early respiratory distress. According to our results only $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ appeared to be suitable indicators.

METHODS

Surgical procedures

Six Yorkshire pigs (about 6 weeks old, 9.5 $\pm$ 0.4 kg body weight) were anesthetized with sodium pentobarbital (30 $mg.kg^{-1}$, i.p.) and mechanically ventilated in supine position on a thermo-controlled operation table at 38°C. A polythene single lumen catheter was inserted through the right common carotid artery into the aortic

arch for measuring arterial blood pressure and sampling of blood. Three catheters were inserted via the right external jugular vein: a Swan-Ganz catheter into the left pulmonary artery for monitoring pulmonary arterial pressure and blood temperature, and for sampling of mixed venous blood; a double walled catheter into the right atrium for injection of cold saline at room temperature during the thermodilution procedures; a four lumen catheter into the superior vena cava for measuring central venous pressure and for infusion of fluids and anesthetics. All catheters for measuring blood pressures were continuously flushed with normal saline (3 $ml.h^{-1}$) containing heparine (10 I.U. ml^{-1}) to avoid clotting in the catheters. A catheter was put into the urinary bladder to avoid retention of urine and to estimate fluid balance as we did in a former study.

After surgery the animals were connected to a volume controlled ventilator (21). Tidal volume (V_T) was adjusted to a P_{aCO2} of 38-42 mmHg during baseline. Other ventilatory settings were: a frequency of 10 per minute, an inspiratory oxygen fraction of 0.6 and an inspiratory to expiratory ratio of 2:3. These ventilatory parameters were kept constant throughout the experiments. Unless mentioned otherwise positive end-expiratory pressure (PEEP) was 2 cmH_2O.

Measurements and data acquisition.

Gas exchange, acid-base and hemoglobin

Oxygen and carbon dioxide tensions in blood and acid-base indices were determined with use of an automatic blood gas analyzer (Radiometer ABL3). Hemoglobin concentration (Hb) and oxygen saturation were determined with use of an oxymeter (Radiometer OSM2). All blood samples were replaced by an equal volume of saline. Inspiratory and mixed expiratory gases, including Helium, were analyzed by a mass spectrometer (Perkin-Elmer, MGA 1100).

Carbon dioxide tension was monitored in the tracheal cannula with use of a capnometer (Hewlett Packard type 47210A). From the expiratory P_{CO2} curve end-tidal carbon dioxide tension (P_{etCO2}) was used for analysis.

Intrapulmonary right-to-left shunt (Q'_s) was calculated as venous admixture (Q'_s/Q'_t) in percentage of cardiac output (Q'_t) according to the equation:

$$Q'_s/Q'_t = 100 \times (C_{cO2}-C_{aO2}) / (C_{cO2}-C_{\bar{v}O2})$$

Oxygen content in arterial (C_{aO2}), mixed venous ($C_{\bar{v}O2}$) and pulmonary end-capillary blood (C_{cO2}) was calculated according to the equation:

$$C_{xO2} = (0.01S_{xO2} \times 1.39Hb) + (0.0031P_{xO2})$$

where x stands for a, $\bar{v}$ and c respectively. S_{xO2} is oxygen saturation in percentage and P_{xO2} oxygen tension in mmHg. Oxygen binding capacity, 1.39 ml oxygen per g Hb,

was used as prescribed by the International Committee for Standardisation in Haematology in 1965. The solubility of oxygen, 0.0031, was expressed in ml per 100 ml blood and per mmHg. C_{xO2} was obtained in ml O_2 per 100 ml blood.

The oxygen saturation of the pulmonary end-capillary blood (S_{cO2}) was derived from the oxygen saturation curve of pig blood and the alveolar oxygen tension (P_{AO2}), as a substitute of pulmonary end-capillary oxygen tension. The equation of the human oxygen saturation curve was adapted to the pig's oxygen saturation curve, based on pig blood data from other experiments in our laboratory. This oxygen saturation curve was similar to the curve described by Bartels (3).
P_{AO2} was derived from the equation:

$$P_{AO2} = P_{IO2} - P_{ACO2} [F_{IO2} + (1-F_{IO2}) / R]$$

where P_{IO2} and F_{IO2} are the inspiratory oxygen tension and fraction respectively. P_{ACO2} is the alveolar carbon dioxide tension, which we assumed to be equal to P_{aCO2}, and R is the respiratory quotient.
Oxygen uptake (V'_{O2}) was derived from:

$$V'_{O2} = F_{iO2} \times V'_I - F_{\bar{E}O2} \times V'_E$$

where V'_I and V'_E are inspiratory and expiratory flow respectively and $F_{\bar{E}O2}$ is the mixed expiratory oxygen fraction.

Physiological dead space (V_D) in percentage of V_T was obtained from:

$$V_D/V_T = 100(P_{aCO2} - P_{\bar{E}CO2})/P_{aCO2}$$

where $P_{\bar{E}CO2}$ is the mixed expiratory carbon dioxide tension.

Pulmonary data

End-expiratory lung volume

We used an open circuit wash-in and wash-out method for helium (He) to obtain values of end-expiratory lung volume (V_{EE}) (18). In the open circuit He wash-in method the lungs were ventilated during 90 sec (15 cycles) with a gas mixture containing about 4 % He. A computer controlled ventilator with two pistons was used, developed in our laboratory (21). During the normal ventilation with one bellows the other bellows was flushed with a 4 % He gas mixture. The open circuit wash-in started when mechanical ventilation was switched at end-expiration to the other bellows. After He was equilibrated between bellows and lungs, the computer controlled ventilator switched back to the first bellows, again at end-expiration, starting the wash-out procedure.

During the wash-in and wash-out procedure V_{EE} was calculated according to the formula:

$$V_{EE} = (\int V' \times F_{I,He}.dt - \int V' \times F_{E,He}.dt) / F_{EE,He}$$

where V' is the airflow rate, $F_{I,He}$ the inspiratory fraction of He, $F_{E,He}$ the expiratory fraction of He and $F_{EE,He}$ the end expiratory He fraction. The He fraction at the end of expiration was assumed to be equal to the fraction that remained in the lungs at the end of the expiration.

Lung compliance

Total compliance of lungs and thorax (C_{rs}) was estimated with use of an inspiratory pause method (5,34). Tracheal pressure ($P_{T,p}$) was measured in the tracheal cannula (gas pressure transducer, type 270, Hewlett Packard). Three inspiratory pauses of 3 sec with insufflation volumes of 6, 12 and 18 $ml.kg^{-1}$ at intervals of two min were inserted during normal ventilation. During these pauses tracheal pressure and thoracic volume decreased gradually. These volume changes during the maneuvers were recorded with use of a mercury cord, which was fixed around the thorax about 5 cm cranial from the sternal xyphoid. The three inflation volumes, mentioned above, served to calibrate the mercury cord. To estimate C_{rs} a third degree polynomial pressure-volume (P-V) curve was fitted through end-expiratory pressure and volume, and the pressures corresponding with the three end-inspiratory volumes. The relationship yielded an approximately linear part between the volumes 4 and 8 $ml.kg^{-1}$. Compliance was derived from this part of the P-V curve (17). Compliance estimates were obtained during baseline conditions and after lavages at a PEEP of 2 cmH_2O.

As an approximation of compliance at the different PEEP levels we used the tidal volume divided by $P_{T,p}$ minus PEEP (C_{dyn}) (7,15,41). We compared the values of C_{dyn} with C_{rs} before lavages, at about 2 h after lavages and at the end of the experiment, which was about 30 min after the PEEP procedures.

Hemodynamic data and oxygen delivery

Arterial blood pressure (P_{aO}), pulmonary arterial blood pressure (P_{pa}) and central venous blood pressure (P_{cv}) were measured continuously with use of Statham transducers (type P23De) and averaged over a ventilatory cycle. Pressures were referred to ambient air pressure and a zero level at the height of the manubrium.

A close correlation between changes in P_{cv} and P_{it} was found in pigs (33). Therefore, we used changes in P_{cv} as substitute for changes in P_{it} to estimate changes in transmural pulmonary arterial pressure ($P_{pa,tm}$) .

Mean cardiac output (Q'_t) was determined by the thermodilution method. Four determinations, equally spread over the ventilatory cycle, were performed in four minutes. The average was used as an estimate of Q'_t (20).

Oxygen delivery (D_{O2}) was calculated according to the equation:

$$D_{O2} = C_{aO2} \times Q'_t$$

The PEEP level, where oxygen delivery was maximal in each individual experiment was normalized, and defined as "best PEEP" (34). For reasons of statistical tests we have reset "best PEEP" to 0 cmH_2O and the other PEEP levels to differences from this level.

Protocol of the experiments

After surgery a stabilisation period of half an hour was inserted. At the end of this period V_{EE} was determined. Next, a baseline period of one hour followed, in which measurements of gas exchange and hemodynamic variables were done. Then, lung lavages were performed. The regime consisted of a set of two lavages at an interval of 5 min followed by another set of two lavages one hour later. During each lavage 35 $ml.kg^{-1}$ saline was washed in and out twice, implicating an interruption of the ventilation of 90 sec. Previously, we developed this regime causing a stable respiratory distress for about 6 h without changing ventilatory settings (16). The results from this study served as control data for the present study.

After lavages the animals were monitored for two hours to evaluate stability. Then, positive end-expiratory pressure (PEEP) was increased from $PEEP_2$ (PEEP of 2 cmH_2O) by steps of 2 cmH_2O up to $PEEP_{14}$. Each step was maintained for half an hour. Blood gas and acid-base values and inspiratory and expiratory gas analyses were performed 10 min and 30 min after the start of each increase in PEEP. V_{EE} and hemodynamic measurements were performed at 20 and 25 min respectively after each change in PEEP. When this PEEP protocol was finished, PEEP was reset in a period of ten minutes to 2 cmH_2O. Twenty minutes later all measurements were repeated to verify whether the initial $PEEP_2$ values were regained.

Statistical analysis

The results were analyzed using the student t-test for paired and unpaired samples, standard repeated measures analysis of variance (SPSS-MANOVA) and Pearson's correlation coefficient analysis; p-values ≤ 0.05 were accepted as statistically significant. Data are presented as mean values $\pm$ 1 sd for those variables that showed a similar reaction in all animals.

RESULTS

Comparison of values before and after lavages at $PEEP_2$

Gas exchange, pulmonary and hemodynamic variables before lavages and at two hours after lavages are summarized in Table 5.1 and compared with the control data. The data of the present group were similar to those of the previous group (16), implying that the present experiments also fulfilled the criteria of respiratory distress (25). The difference between C_{dyn} and C_{rs} was not changed by the lavages. Oxygen uptake was the same before and after the lavages.

PEEP

Gas exchange variables

PEEP increased arterial oxygen tension (P_{aO2}) from 55 ± 14 mmHg at $PEEP_2$ (=2 h after lavages) to 218 ± 94 mmHg at $PEEP_{14}$ ($p<0.01$, Fig. 5.1a). Oxygen saturation (S_{aO2}) was increased from 61 ± 23 % at $PEEP_2$ to 98 ± 2 % at $PEEP_{14}$ ($p<0.001$, Fig. 5.1b). Mixed venous oxygen tension ($P_{\bar{v}O2}$) increased from 35 ± 5 mmHg at $PEEP_2$ to a maximum at about $PEEP_6$, where $P_{\bar{v}O2}$ was on average 45 ± 3 mmHg ($p<0.01$, Fig. 5.1c). From $PEEP_6$ up to $PEEP_{10}$ $P_{\bar{v}O2}$ decreased hardly to 44 ± 3 mmHg, but above $PEEP_{10}$ slightly more to 39 ± 5 mmHg at $PEEP_{14}$ ($p<0.01$). $P_{\bar{v}O2}$ had a linear and quadratic trend from $PEEP_2$ to $PEEP_{14}$ ($p<0.01$, SPSS MANOVA). Mixed venous oxygen saturation ($S_{\bar{v}O2}$) increased from 28 ± 14 % at $PEEP_2$ to 47 ± 7 % at $PEEP_6$ ($p<0.01$, Fig. 5.1d). Above $PEEP_{10}$ $S_{\bar{v}O2}$ decreased to 37 ± 12 % at $PEEP_{14}$ ($p<0.01$ with respect to the value at $PEEP_6$). $S_{\bar{v}O2}$ had a linear and quadratic trend (SPSS MANOVA, $p<0.01$). Venous admixture (Q'_s/Q'_t) decreased from 59 ± 19 % at $PEEP_2$ to 7 ± 6 % at $PEEP_{14}$ ($p<0.01$, Fig. 5.1e). Oxygen uptake (V'_{O2}) was not changed by PEEP.

Arterial carbon dioxide tension (P_{aCO2}) decreased from 66 ± 13 mmHg at $PEEP_2$ to 53 ± 9 mmHg at $PEEP_{14}$ ($p<0.01$, Fig. 5.2a), whereas the control value significantly increased from 64 ± 9 mmHg to 94 ± 10 mmHg. The difference between arterial and end-tidal carbon dioxide (P_{aCO2}-P_{etCO2}) decreased by PEEP from 23 ± 10 mmHg at $PEEP_2$ to 7 ± 5 mmHg at $PEEP_{14}$ ($p<0.01$, Fig. 5.2b). The decrease in P_{aCO2}-P_{etCO2} by PEEP was greater between $PEEP_2$ and $PEEP_{10}$ than it was above $PEEP_{10}$. The control data revealed an increase of P_{aCO2}-P_{etCO2} from 20 ± 6 mmHg to 34 ± 9

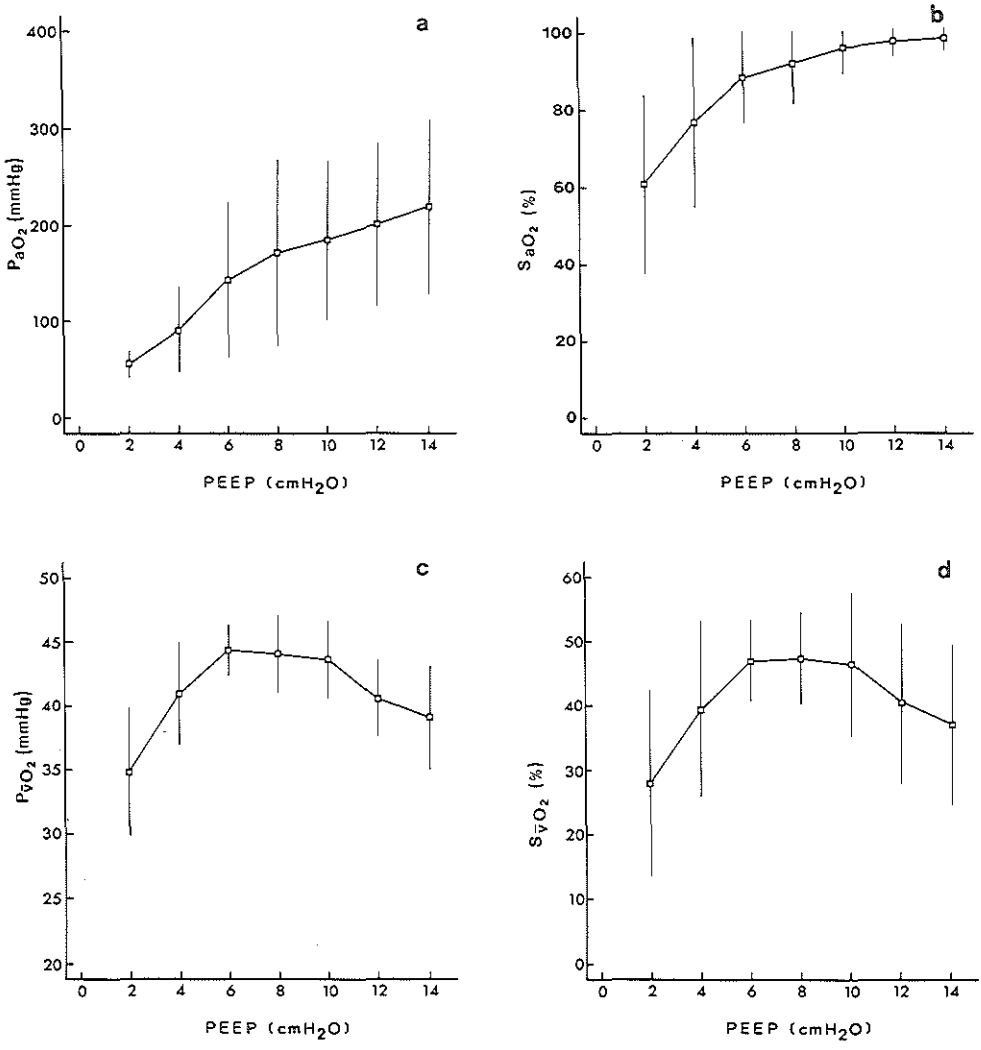

Fig. 5.1. Data related to P_{O2}.

Variables are plotted against postitive end-expiratory pressure (PEEP). Vertical bars: sd. a. arterial oxygen tension, b. arterial oxygen saturation, c. mixed venous oxygen tension, d. mixed venous oxygen saturation, e. venous admixture. e: next page.

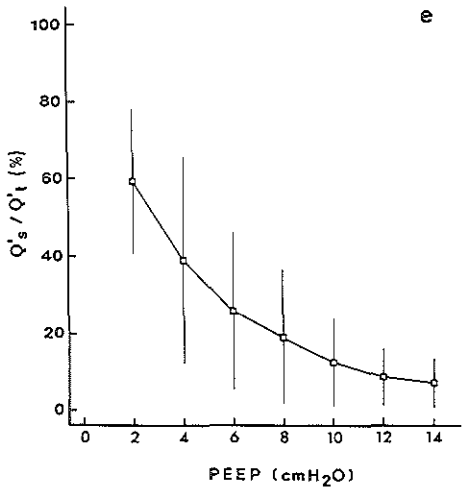

Fig. 5.1e.
Venous admixture versus PEEP. Vertical bars:sd.

mmHg ($p<0.05$). Physiological dead space (V_D/V_T) decreased from 56 ± 6 % at $PEEP_2$ to 43 ± 7 % at $PEEP_{14}$ ($p<0.01$, Fig. 5.2c), whereas in the control group V_D/V_T increased from 55 ± 5 % to 68 ± 3 %. pH increased from 7.25 ± 0.09 at $PEEP_2$ to 7.31 ± 0.08 at $PEEP_{14}$ ($p<0.01$). At the end pH was not different from the value before PEEP, whereas pH in the previous study of the control group was decreased from 7.26 ± 0.07 at 2 h after lavages to 7.11 ± 0.08 at the end. HCO_3^- concentration at 2 h was not changed by the lavages and was not changed by PEEP.

Pulmonary variables

End-expiratory lung volume , $V_{EE\text{-}in}$, increased from 12 ± 2 $ml.kg^{-1}$ at $PEEP_2$ to 46 ± 9 $ml.kg^{-1}$ at $PEEP_{14}$ ($p<0.001$). The end-expiratory lung volume, $V_{EE\text{-}out}$, increased from 10 ± 3 $ml.kg^{-1}$ at $PEEP_2$ to 43 ± 9 $ml.kg^{-1}$ at $PEEP_{14}$ ($p<0.001$, Fig. 5.3a). Both $V_{EE\text{-}in}$ and $V_{EE\text{-}out}$ had a linear and quadratic trend (SPSS MANOVA, $p<0.01$). $V_{EE\text{-}in}$ correlated strongly with $V_{EE\text{-}out}$ ($r=0.99$, $p<0.001$).

Tracheal pressure at peak-insufflation ($P_{T,p}$) remained about the same between $PEEP_2$, where its value was 40 ± 4 cmH_2O, and $PEEP_8$. Then, $P_{T,p}$ increased to 52 ± 6 cmH_2O at $PEEP_{14}$ ($p<0.001$ with respect to $PEEP_2$ and $PEEP_8$). $P_{T,p}$ between $PEEP_2$ and $PEEP_{14}$ had a linear and quadratic trend

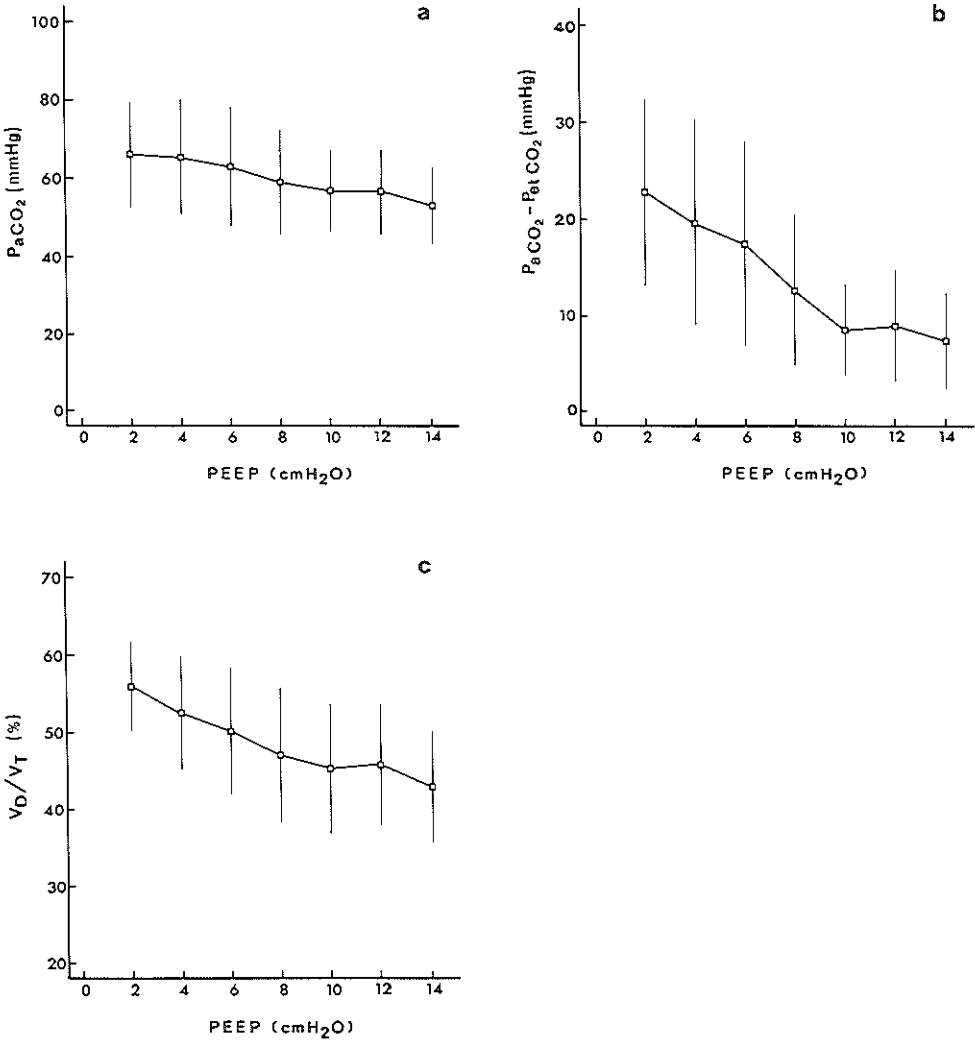

Fig. 5.2. Data related to P_{CO2}.
Variables are plotted against PEEP. Vertical bars: sd. a. arterial carbon dioxide tension, b. arterial minus end-tidal carbon dioxide tension, c. physiological dead space.

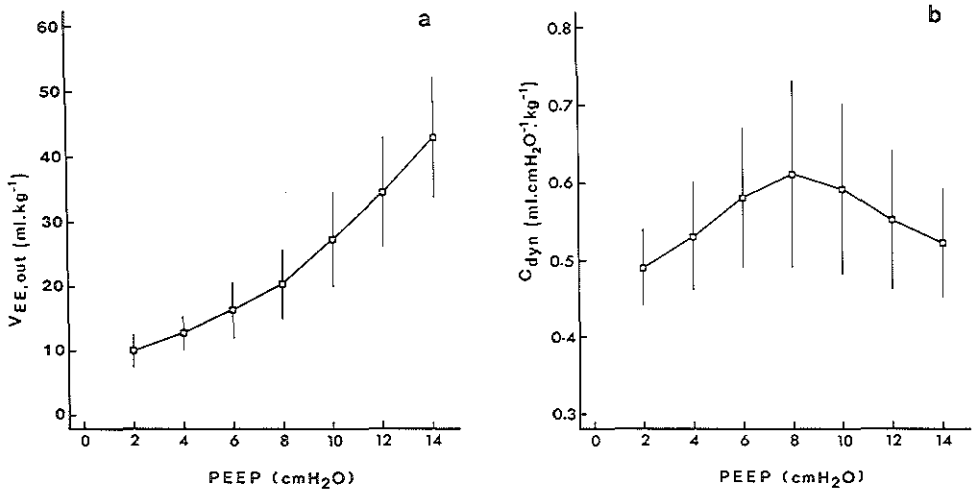

Fig. 5.3. Lung mechanics.
Variables are plotted against PEEP. Vertical bars: sd. a. end-expiratory lung volume (helium open out-wash method), b. dynamic lung compliance.

($p<0.01$, SPSS MANOVA).

Dynamic lung compliance (C_{dyn}) increased from 0.49 ± 0.06 ml. cmH_2O^{-1} at $PEEP_2$ to 0.58 ± 0.09 ml.cmH_2O^{-1} at $PEEP_8$ ($p<0.01$, Fig. 5.3b). Between $PEEP_8$ and $PEEP_{12}$ C_{dyn} did not change significantly. Then, C_{dyn} decreased to 0.52 ± 0.07 ml.cmH_2O at $PEEP_{14}$ ($p<0.05$ with respect to $PEEP_8$, but not different from $PEEP_2$).

Hemodynamic variables

Cardiac output (Q'_t) decreased from 2.5 ± 1.0 ml.s^{-1}.kg^{-1} at $PEEP_2$ to 1.2 ± 0.3 ml.s^{-1}.kg^{-1} at $PEEP_{14}$ (linear trend $p<0.05$, SPSS MANOVA; Fig. 5.4a).

Aortic pressure (P_{ao}) decreased from 95 ± 17 mmHg at $PEEP_2$ to 65 ± 17 mmHg at $PEEP_{14}$ (linear trend $p<0.05$, SPSS MANOVA; Fig. 5.4b).

Pulmonary arterial pressure (P_{pa}) decreased from 32 ± 3 mmHg at $PEEP_2$ to 21 ± 2 mmHg at $PEEP_{10}$ ($p<0.01$, Fig. 5.4c). The further rise up to $PEEP_{14}$, where its value was 23 ± 2 mmHg ($p<0.01$ with respect to $PEEP_2$), was not significant. P_{pa} had a significant linear and quadratic trend ($p<0.01$, SPSS MANOVA).

Transmural pulmonary arterial pressure ($P_{pa,tm}$) was decreased with 11 ±

1 mmHg at $PEEP_8$ with respect to the value at $PEEP_2$ ($p<0.01$, Fig. 5.4d). From $PEEP_8$ up to $PEEP_{14}$ $P_{pa,tm}$ did not change significantly.

Central venous pressure (P_{cv}) increased from 0.9 ± 1.1 mmHg at $PEEP_2$ to 4.1 ± 1.4 mmHg at $PEEP_{14}$ (linear trend $p<0.001$, SPSS MANOVA; Fig. 5.4e).

Heart rate (HR) increased linearly from 189 ± 50 beats.min^{-1} at $PEEP_2$ to 250 ± 23 beats.min^{-1} at $PEEP_{14}$ ($p<0.05$, Fig. 5.4f).

Oxygen delivery

Oxygen delivery (D_{O2}) increased from 22 ± 5 ml.s^{-1}.kg^{-1} at $PEEP_2$ to 27 ± 7 ml.s^{-1}.kg^{-1} at $PEEP_6$ ($p<0.05$, Fig. 5.5). Above $PEEP_6$ D_{O2} decreased to 20 ± 4 ml.s^{-1}.kg^{-1} at $PEEP_{14}$ ($p<0.01$ with respect to $PEEP_6$). Repeated measures analysis of variance revealed a linear and quadratic trend ($p<0.05$, SPSS MANOVA).

For each individual animal the maximum value of oxygen delivery was estimated ("best PEEP"). "Best PEEP" was on average 6 cmH_2O. In the individual animals "best PEEP" was at $PEEP_4$ (n=1), $PEEP_6$ (n=4) and $PEEP_{10}$ (n=1). Above "best PEEP" D_{O2} decreased in all animals when a higher PEEP was applied. The mean value of D_{O2} at "best PEEP" was 28 ± 6 ml.s^{-1}.kg^{-1}. D_{O2} at 4 cmH_2O below "best PEEP" was 21 ± 6 ml.s^{-1}.kg^{-1} ($p<0.05$ with respect to "best PEEP"). At 4 cmH_2O above "best PEEP" D_{O2} was 24 ± 5 ml.s^{-1}.kg^{-1} ($p<0.05$ with respect to "best PEEP", Table 5.2).

"Best PEEP" coincided with the maximum of $P_{\bar{v}O2}$, except for one experiment where the maximum value of $P_{\bar{v}O2}$ was 2 cmH_2O below "best PEEP".

The mean values of $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ had approximately the same pattern of changes as those of D_{O2} (Table 5.2). In the other variables the pattern was different.

The correlation between several variables and D_{O2} in the individual animals is presented in Table 5.3. $P_{\bar{v}O2}$ had a significant correlation with D_{O2} in five and $S_{\bar{v}O2}$ in four animals. The other variables did not correlate with D_{O2}.

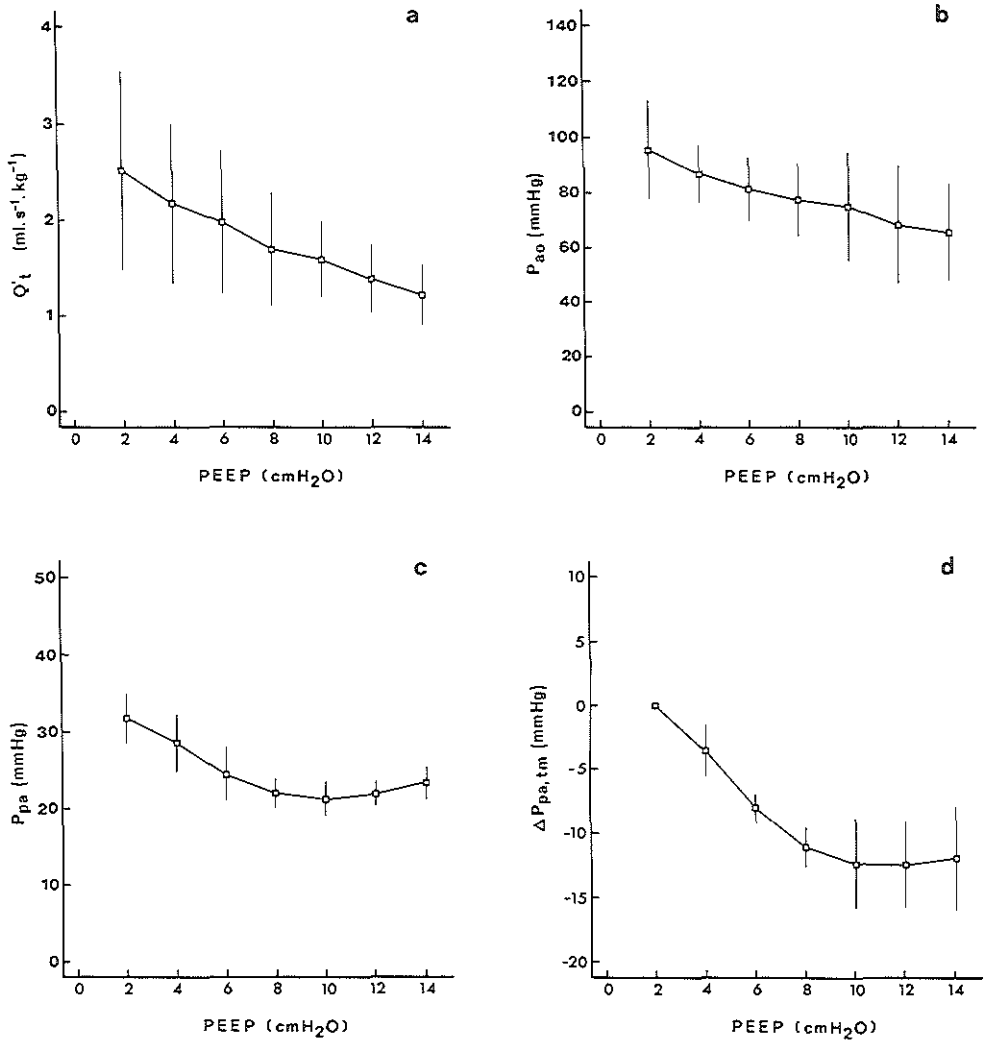

Fig. 5.4. Hemodynamic data.
Variables are plotted against PEEP. Vertical bars: sd. a. cardiac output, b. aortic pressure, c. pulmonary arterial pressure, d. changes in transmural pulmonary arterial pressure, e. central venous pressure, f. heart rate (e and f: next page).

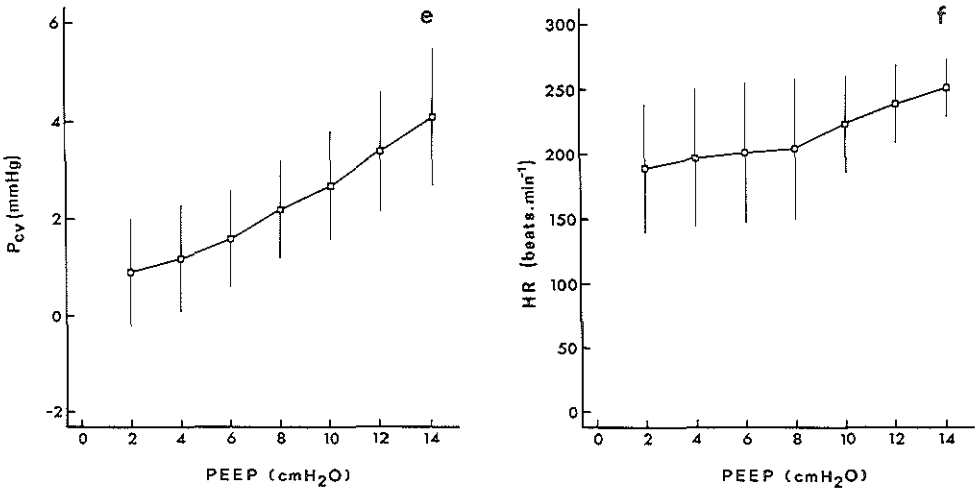

Fig. 5.4e and f.

After PEEP

Gas exchange, pulmonary and hemodynamic variables at the end of the experiments were summarized in the last column of Table 5.1 and were compared with the values before PEEP and with the control data of our previous study at the same time after lavage.

Two animals died shortly after PEEP was returned to $PEEP_2$ and before the estimation of cardiac output was performed.

P_{aO2}, S_{aO2} and P_{vO2} were not significantly different from the values before the application of PEEP. However, compared with the control data they were increased. After PEEP Q'_s/Q'_t, P_{aCO2}, V_D/V_T, P_{aCO2}-P_{etCO2}, P_{ao} and P_{pa} were not different from the values before PEEP, but they were lower than the control values. pH after PEEP was not different from the value at 2 h after lavages. However, pH after PEEP was higher than in the control group. $P_{T,p}$ after the PEEP procedures was decreased compared with the value before these procedures, and was also lower than $P_{T,p}$ at the corresponding time in the control study. C_{dyn} was increased after PEEP compared with the value before PEEP and compared with its corresponding control value. The difference between C_{dyn} and C_{rs} at the end was the same as before the PEEP procedures. At the end of the experiments HR was not different from that in the control group, in which HR increased during the sixth hour after lavage (Table 5.1).

Table 5.1. Respiratory and hemodynamic data for the control and PEEP group in baseline, at 2 hours after lavages and at the end of the experiments.

		Baseline	2 h post lavage	End
P_{aO2}(mmHg)	P	298 ± 15	55 ± 14**	95 ± 49#
	C	306 ± 26	55 ± 13**	46 ± 6
S_{aO2}(%)	P	99 ± 1	61 ± 23**	80 ± 23#
	C	99 ± 1	60 ± 21**	47 ± 10
$P_{\bar{v}O2}$(mmHg)	P	48 ± 3	34 ± 5**	44 ± 6#
	C	49 ± 4	34 ± 9**	31 ± 7
$S_{\bar{v}O2}$ (%)	P	64 ± 7	28 ± 14**	37 ± 21
	C	66 ± 7	31 ± 11**	20 ± 9
Q'_s/Q'_t(%)	P	6 ± 2	59 ± 18**	21 ± 10#
	C	6 ± 1	55 ± 19**	67 ± 7
P_{aCO2}(mmHg)	P	43 ± 2	66 ± 13**	63 ± 9##
	C	40 ± 2	64 ± 9**	94 ±10**
$P_{aCO2} - P_{etCO2}$ (mmHg)	P	3 ± 2	22 ± 10**	21 ± 18#
	C	2 ± 1	20 ± 6**	34 ± 9*
V_D/V_T (%)	P	32 ± 4	56 ± 6**	51 ± 7##
	C	34 ± 4	55 ± 5**	68 ± 3**
pH	P	7.45 ± 0.02	7.25 ± 0.09**	7.24 ± 0.09#
	C	7.47 ± 0.03	7.26 ± 0.07**	7.11 ± 0.08*
HCO_3^- (mmol.l^{-1})	P	29.6 ± 0.9	28.1 ± 1.2	27.7 ± 1.3
	C	29.0 ± 1.3	27.5 ± 1.6	27.3 ± 2.5
Hb(mmol.l^{-1})	P	5.8 ± 0.9	6.2 ± 0.2	7.0 ± 0.9
	C	5.8 ± 0.9	6.6 ± 1.2 *	7.2 ± 0.4
$V_{EEopen\text{-}in}$(ml.kg^{-1})	P	21.5 ± 1.1	11.5 ± 2.2**	14.9 ± 2.4
	C	21.7 ± 1.4	-	12.7 ± 2.1**

$V_{EEopen\text{-}out}$(ml.kg^{-1})	P	21.3 ± 1.5	10.0 ± 2.5**	12.8 ± 4.7*
	C	21.5 ± 0.9	-	11.8 ± 2.9**
$P_{T,p}$ (cmH_2O)	P	19 ± 3	40 ± 4**	35 ± 6$^{*,\#}$
	C	19 ± 3	39 ± 3**	47 ± 2**
C_{dyn} (ml.cmH_2O^{-1}.kg^{-1})	P	1.08 ± 0.11	0.49 ± 0.06**	0.62 ± 0.13$^{*,\#}$
	C	1.08 ± 0.13	0.50 ± 0.03**	0.41 ± 0.04
C_{rs}(ml.cmH_2O^{-1}.kg^{-1})	P	1.6 ± 0.1	0.8 ± 0.2**	0.9 ± 0.2
	C	1.7 ± 0.2	0.7 ± 0.2**	0.6 ± 0.1
Q'_t(ml.s.$^{-1}$.kg^{-1})	P	2.2 ± 0.3	2.5 ± 1.0	1.6 ± 1.3
	C	2.1 ± 0.1	2.4 ± 0.5	2.2 ± 0.6
D_{O2}(ml.s^{-1}.kg^{-1})	P	30 ± 7	22 ± 5*	28 ± 4
	C	29 ± 6	21 ± 5*	21 ± 6
V'_{O2} (ml.s^{-1}.kg^{-1})	P	0.10 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
	C	0.13 ± 0.01	0.13 ± 0.03	0.13 ± 0.01
R_s (mmHg.s.kg.ml^{-1})	P	44 ± 6	42 ± 9	45 ± 6$^{\#}$
	C	39 ± 10	42 ± 9	33 ± 7
P_{ao}(mmHg)	P	96 ± 10	95 ± 17	70 ± 18
	C	92 ± 14	89 ± 15	75 ± 21
P_{pa}(mmHg)	P	11 ± 2	32 ± 3**	24 ± 5
	C	12 ± 1	31 ± 2**	30 ± 6
P_{cv}(mmHg)	P	0.2 ± 0.8	0.9 ± 1.1*	0.3 ± 1.5
	C	0.6 ± 1.6	0.8 ± 1.0	0.7 ± 1.8
HR (beats.min^{-1})	P	184$^{\#}$ ± 23	189 ± 50	217 ± 25
	C	144 ± 24	164 ± 47	213 ± 37

P = PEEP group, C = Control group. *: $p < 0.05$ and **: $p < 0.01$ compared to the previous column. $^{\#}$: $p < 0.05$ and $^{\#\#}$: $p < 0.01$ compared to the control group. V_{EE} in the control group compared to baseline. C_{rs} in the control group at 2 h post-lavage: n=4. Q'_t and D_{O2} at the end in the PEEP group: n=4.

Table 5.2. Respiratory and hemodynamic data after lavages with respect to best PEEP.

	-4 cm H_2O	-2 cm H_2O	0 cm H_2O	+2 cm H_2O	+4 cmH_2O
D_{O2}	21 ± 6**	24 ± 7**	28 ± 6	26 ± 5*	24 ± 5**
P_{aO2}	89 ± 88	103 ± 96	135 ± 87	165 ± 94*	187 ± 88*
S_{aO2}	61 ± 28*	73 ± 19*	88 ± 11	92 ± 10*	95 ± 6*
C_{aO2}	10 ± 4	12 ± 3**	14 ± 2	15 ± 2*	16 ± 2*
$P_{\bar{v}O2}$	35 ± 6**	40 ± 5*	45 ± 3	45 ± 2	43 ± 3*
$S_{\bar{v}O2}$	28 ± 18**	37 ± 13**	48 ± 9	48 ± 9	42 ± 12
Q'_s/Q'_t	56 ± 33*	43 ± 25*	27 ± 19	19 ± 1**	12 ± 11**
P_{aCO2}	66 ± 16	66 ± 15	63 ± 15	59 ± 12	57 ± 9
P_{aCO2}-P_{etCO2}	22 ± 12**	20 ± 11	17 ± 11	13 ± 8	9 ± 4
V_D/V_T	55 ± 9*	52 ± 9*	49 ± 10	47 ± 8	45 ± 7
V_{EEin}	13 ± 5	16 ± 8@	19 ± 10	24 ± 13**	29 ± 15**
V_{EEout}	12 ± 7*	15 ± 7*	19 ± 10	23 ± 12**	28 ± 13**
$P_{T,p}$	40 ± 5	40 ± 6	39 ± 5	41 ± 6	44 ± 6*
C_{dyn}	0.51 ± 0.10*	0.55 ± 0.12	0.59 ± 0.10	0.58 ± 0.10	0.57 ± 0.10
Q'_t	2.5 ± 1.2	2.2 ± 0.9	2.0 ± 0.7	1.8 ±0.5**	1.5 ±0.4**
P_{ao}	97 ± 18	88 ± 10	85 ± 15	78 ± 14**	71 ± 14**
P_{pa}	31 ± 5	29 ± 4	26 ± 2	22 ± 2**	21 ± 3**
P_{cv}	1.3 ± 1.3*	1.2 ± 1.5*	1.7 ± 1.5	2.3 ±1.7**	3.0 ±1.7**
HR	200 ± 48	200 ± 51	212 ± 43	218 ± 44	223 ± 45

Mean ± sd; best PEEP was reset to 0 cmH_2O; -4 cmH_2O: n=5; @ : n=5. *:$p < 0.05$ compared to value at best PEEP; **:$p < 0.01$ compared to value at best PEEP. D_{O2}, oxygen delivery in ml.s^{-1}.kg^{-1}; P_{aO2}, arterial oxygen tension in mmHg; S_{aO2}, arterial oxygen saturation in %; C_{aO2}, oxygen content in ml.100 ml^{-1}; $P_{\bar{v}O2}$, mixed venous oxygen tension in mmHg; $S_{\bar{v}O2}$, mixed venous oxygen saturation in %; Q'_s/Q'_t, venous admixture in %; P_{aCO2}, arterial carbon dioxide tension in mmHg; P_{aCO2}-P_{etCO2}, arterial minus end-tidal carbon dioxide tension in mmHg; V_D/V_T, physiological dead space in %; $V_{EE\text{-}in}$, end-expiratory lung volume (He wash-in) in ml.kg^{-1}; $V_{EE\text{-}out}$, end-expiratory lung volume (He wash-out) in ml.kg^{-1}; $P_{T,p}$, peak tracheal pressure in cmH_2O; C_{dyn}, dynamic respiratory compliance in ml.cmH_2O^{-1}.kg^{-1}; Q'_t, cardiac output in ml.s^{-1}.kg^{-1}; P_{ao}, arterial blood pressure in mmHg; P_{pa}, pulmonary arterial pressure in mmHg; P_{cv}, central venous pressure in mmHg; HR, heart rate in beats per min.

Table 5.3. Correlation (r) with oxygen delivery.

exp (no.)	$P_{\bar{v}O2}$ (mmHg)	$S_{\bar{v}O2}$ (%)	Q'_s/Q'_t (%)	V_D/V_T (%)	P_{aCO2}-P_{etCO2} (mmHg)	$V_{EE\text{-}out}$ ($ml.kg^{-1}$)	C_{dyn} ($ml.cmH_2O^{-1}.kg^{-1}$)
1	0.90 (0.01)*	0.97 (0.001)**	0.10 (0.99)	0.21 (0.92)	-0.93 (0.06)	-0.73 (0.09)	-0.25 (0.74)
2	0.94 (0.01)*	0.87 (0.01)*	-0.79 (0.10)	-0.57 (0.30)	-0.91 (0.03)*	0.05 (0.90)	0.87 (0.03)*
3	0.93 (0.02)*	0.90 (0.01)*	-0.66 (0.21)	-0.45 (0.44)	-0.61 (0.28)	-0.17 (0.76)	0.87 (0.054)
4	0.78 (0.12)	0.50 (0.31)	-0.02 (0.97)	0.19 (0.76)	-0.88 (0.051)	-0.97 (0.001)**	0.78 (0.12)
5	0.76 (0.03)*	0.90 (0.005)**	0.09 (0.88)	0.31 (0.61)	-0.64 (0.48)	-0.75 (0.051)	0.42 (0.48)
6	0.81 (0.03)*	0.62 (0.14)	-0.46 (0.31)	-0.72 (0.16)	-0.38 (0.53)	0.55 (0.19)	0.28 (0.64)
score	**5**	**4**	**0**	**0**	**1**	**1**	**0**

In the last row the score of significant correlations is given for each variable. p-values in brackets. * $p<0.05$; ** $p<0.01$. exp (no.): experiment number.

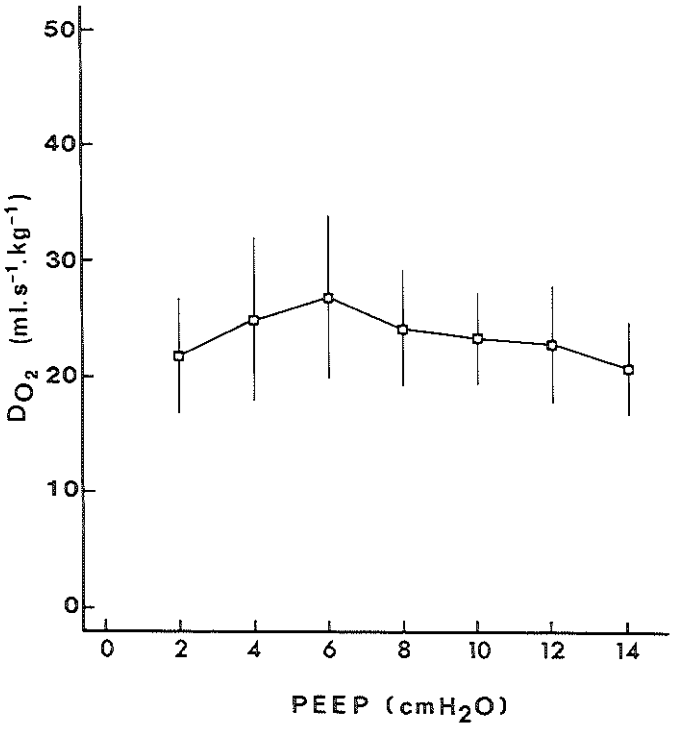

Fig. 5.5. Oxygen delivery plotted against PEEP.
Vertical bars: sd.

DISCUSSION

Lavage model

In a previous study we developed a model of respiratory distress by comparing different regimes of lung lavages with saline (16). The lavage model was stable for 6 h and fullfilled the criteria of moderate to severe human RDS (25), and was characterized by edema, atelectasis and vasoconstriction (17). From that study we concluded that the lavage regime, consisting of a pair of lavages within five minutes, followed by another pair of lavages after one hour, was the most suitable regime for studies on basic mechanisms and therapeutic interventions in early respiratory distress.

We did not find any differences in gas exchange, pulmonary and

hemodynamic variables between our present and previous group in baseline and during the period of 2 h after lavages (Table 5.1). Thus, we concluded 1) that the animals of the present study reacted similarly to lung lavages as the animals in our previous study and 2) that our data in the previous group at the same time after lavages could serve as control data.

The differences between C_{dyn} and C_{rs} in baseline conditions, after lavage and at the end of the experiments (Table 5.2) will have been mainly due to airflow and airway resistance (5,35). The difference between both variables did not change by lavage. Because we kept airflow constant, we concluded from this constant difference that airflow resistance was the same under baseline conditions, after lavage and at the end.

Effects of PEEP

Gas exchange and pulmonary data

The increase in PEEP caused an increase in V_{EE}, which probably was the main reason for the rise in P_{aO2} and S_{aO2}. Similar responses were found in the early stages of human RDS (9,22). Sandhar et al. (29) concluded from model calculations that the rise in P_{aO2} with PEEP after lavages could not have been caused by a decrease in cardiac output.

$P_{\bar{v}O2}$ increased with PEEP up to $PEEP_6$ due to an increase in oxygen delivery at constant oxygen uptake. Above $PEEP_6$ $P_{\bar{v}O2}$ decreased (Fig. 5.1c). The cause of this decrease in $P_{\bar{v}O2}$ was undoubtedly the decrease in oxygen delivery due to the decrease in cardiac output (Figs. 4a and 5), because oxygen uptake was constant. These results are in agreement with those in other reports (23,30,39). A decrease in venous admixture was attributed to a decrease in cardiac output (23). We confirmed this relationship (Figs. 1e and 4a). The continuing increase in P_{aO2} at higher PEEP, despite the decrease in $P_{\bar{v}O2}$ (Figs. 1a and 1c), can be attributed to the presence of lung units with ventilation-perfusion ratios above 1.0 (10,40).

Physiological dead space decreased up to $PEEP_{14}$ (Fig. 5.2c). At the highest levels of PEEP, we expected physiological dead space to increase as reported by Suter et al. (34). We attributed the fall in V_D/V_T in our conditions of early respiratory distress to a better distribution of pulmonary blood flow. Pulmonary vessels might have dilated in areas, which became better ventilated. Additional evidence for a better distribution of pulmonary blood flow with respect to ventilation is the fall in venous admixture (Fig. 5.1e). The difference with Suter's results may be explained by the more advanced stage of respiratory distress of the patients investigated by him. When vessels become

hypertrophic in a later stage of distress an immediate reaction on an increase in alveolar oxygen tension will be inhibited (36). It needs further studies to conclude whether a decrease in V_D/V_T at increasing PEEP is an indicator of the stage of respiratory distress.

The increase in pH by PEEP was due to the decrease in P_{aCO2} reflecting less respiratory acidosis, because bicarbonate concentration remained constant.

Suter et al. (35) found a close correlation between C_{dyn} and C_{rs} at different levels of PEEP. Therefore, we used C_{dyn} to indicate changes in respiratory system compliance during the PEEP procedures. C_{dyn} increased slightly up to $PEEP_8$ (Fig. 5.3b), which was 2 cmH_2O above the averaged maximum of D_{O2}. C_{dyn} remained at this level up to $PEEP_{12}$, above which it decreased slightly. The increase in C_{dyn} in our study was probably the result of recruitment of previously collapsed alveoli and prevention of terminal airway collapse (15,34). The decrease in C_{dyn} above $PEEP_8$ was presumably caused by overdistension.

Hemodynamic effects

In our study the decreases in Q'_t and P_{ao} and the increase in P_{cv} were linear with the increase in PEEP (Figs. 5.4a, 5.4b and 5.4e). The linear decrease in Q'_t was different from the non-linear decrease when PEEP was applied as a ramp in healthy pigs (32). In this latter study the decrease in Q'_t was attributed to a combination of a rise in P_{cv}, compensating activity of baroreflexes elicited by the decrease in P_{ao} and a negative effect of stretch reflexes by the lung inflation (33). Presumably, this negative effect of the lung stretch reflex had less effect in our lavage pigs due to less compliant lung tissue. In agreement with this supposition is the increase in heart rate with PEEP (Fig. 5.4f), whereas Schreuder et al. observed a decrease between $PEEP_3$ and $PEEP_{10}$ (33).

We did not calculate pulmonary vascular resistance because we assumed that two types of flow resistances in the pulmonary circulation, the Poiseuille resistance and the Starling resistor, existed during continuous positive pressure ventilation (38).

PEEP applied in early respiratory distress could have changed pulmonary arterial pressure by a decrease in cardiac output and by vasodilation of pulmonary arteries. The decrease in P_{pa} from 32 mmHg to 21 mmHg was smaller (34 %) than the fall in cardiac output (52 %). However, we suppose that the decrease in the pressure gradient over the arterial part of the pulmonary circulation was larger, because PEEP will have increased pulmonary capillary pressure. Whenever a dilation of the muscular pulmonary arteries occurred, it presumably was a slight one.

After PEEP

About half an hour after the PEEP procedures when PEEP was again at $PEEP_2$ a fatal circulatory shock developed in two animals. This could be due to insufficient oxygen delivery to some tissues at the highest level of PEEP. However, it might also be an effect of the lavages per se, because the animals of our previous control study, where no interventions were done, started to deteriorate in the sixth hour after lavage (16).

After PEEP the slightly larger V_{EE} and the significantly better values of gas exchange variables, as P_{aO2}, S_{aO2} and P_{aCO2} as well as the higher value of C_{dyn} (Table 5.1) were presumably due to an improvement of ventilation-perfusion inhomogeniety and some decrease in atelectasis.

Optimal PEEP

In all individual experiments a maximum in D_{O2} was found as in the averaged data (Fig. 5.5). The largest rise in S_{aO2} up to $PEEP_6$ was in agreement with the patient study by Suter et al (34). Above $PEEP_6$ S_{aO2} could hardly increase, because S_{aO2} was almost 100 %. Therefore, above $PEEP_6$ the continuous decrease in Q'_t was the main reason for the decrease in D_{O2}. Only a few authors also reported a maximum in oxygen transport (34,37), but others did not find an optimum (4,6,22,24,26,41). These different findings might be due to different stages of ARDS in the patients investigated in the different studies. It was suggested that in the later stages of RDS V_{EE} cannot be increased by PEEP, due to structural alterations in the lung architecture (36).

The effects of PEEP in animals after lung lavages have been studied (8,27,28,29), but "best PEEP" was not analyzed.

From the averaged values of Table 5.2 we concluded that the best indicators of "best PEEP" appeared to be $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$. The correlation of $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ with D_{O2} in the individual experiments was in agreement with this provisional conclusion, although not in all cases those correlations were significant. In our experiments oxygen uptake remained constant. A change in oxygen uptake will result in a change in $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$. Therefore, monitoring of metabolic rate will be needed when $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ are used as indicators of "best PEEP".

We could not confirm any reliability of other indicators of "best PEEP" in this model of early respiratory distress.

Conclusions

By increasing PEEP in lavage induced respiratory distress P_{aO2} and V_{EE} were increased and P_{aCO2}, V_D/V_T and hemodynamic variables were decreased with increasing levels of PEEP. It needs further studies to test whether a decrease in physiological dead space at increasing levels of PEEP is an indicator of an early stage of respiratory distress.

Each individual animal had a PEEP level, where oxygen delivery was maximal, "best PEEP". "Best PEEP" in our lavage model of respiratory distress coincided with the highest level of $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$. We could not confirm such a coincidence with other variables. Presumably, the results found in early respiratory distress do not count for later stages. We suggest that $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ could be usefull indicators of "best PEEP" in patients with early respiratory distress and constant metabolic rate to adjust PEEP to an optimal level.

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CHAPTER VI

FAT EMBOLISM AND OLEIC ACID MODEL

The problem of fat embolism has been studied for more than a century. The first description of this phenomenon was in 1861 by Zenker (92), who observed fat droplets in the lung capillaries of a railroad worker after a thoracoabdominal crush injury. Since the first clinical diagnosis was established in 1873 by Bergmann (7) in a patient with a fracture of the distal femur, numerous studies on fat embolism have been published. Scuderi (78) mentioned in a review article a total number of 600 references up to 1941.

Clinical aspects of fat embolism

Nearly after all lower extremity and pelvic trauma, fat embolization seems to occur (66). However, in most cases this remains subclinical (53). Sometimes a mild arterial hypoxemia is the only laboratory abnormality in subclinical fat embolization (26).

Clinical signs and symptoms of the fat embolism syndrome (FES) are seen in 0.5 % to 2 % of patients with long bone fractures and in 5 % to 10 % of patients with multiple fractures associated with pelvic injuries (26,60). These symptoms are predominantly those of ARDS as described in chapter I.

Usually there is some delay between the trauma and the beginning of the syndrome. It was observed in 75 patients with fat embolism, that 23% showed symptoms within the first 12 hours after injury, 60% within 36 hours and over 90% within 48 hours (81). After this variable interval, progressive respiratory failure will develop, resulting in arterial hypoxemia. The arterial hypoxemia may already be present before the onset of symptoms of respiratory failure (26,76). Petechial rash, coagulation disturbances leading to disseminated intravascular coagulation (9,68) and involvement of the central nervous system causing desorientation, delirium and even coma may complicate the clinical picture (26,78). Mortality of this syndrome approximated 10 % to 20 % (60,69). It has been estimated that more than 5000 deaths annually are the result of the fat embolism syndrome in the United States (59).

Pathogenesis and pathophysiology of fat embolism syndrome

The pathogenesis of the fat embolism syndrome is still not completely known. However, research in the last 35 years has provided more insight and understanding of the pathophysiological mechanisms involved in the development of the fat embolism syndrome.

Origin of the fat

Most investigators assume that embolic fat after trauma is derived from the bone marrow depots of the body (13,26,44,82). This is in accordance with the classical mechanical theory, originally described by Gauss in 1924 (23), in which the fat was claimed to be originated from bone marrow entering the fragile medullary venous sinuses after trauma. Any movement of a fractured bone may cause a new number of fat emboli.

Another theory about the origin of the fat emboli is the physico-chemical theory (51,52). According to this theory, alterations in lipoprotein stability in blood may occur after injury, leading to coalescense of lipoproteins to form gross fat droplets that subsequently enter the lungs. It was suggested that secretion of adrenal catecholamines and corticosteroids might be an important factor in the initiation of this proces, as high doses of steroids in rabbits could generate pulmonary fat embolism (52). Some authors found a cholesterol fraction in emboli after autopsy of patients with FES, which was ten-fold greater than that of marrow fat (34). This result could not be reproduced by others (22).

Lipase

Peltier and coworkers emphasized the importance of an enzyme, lipase, in the pathogenesis of traumatic fat embolism (62). They suggested that neutral bone marrow fat, once filtered by the lung capillaries, was hydrolyzed to fatty acids, which cause a chemical disruption of the endothelium cells, leading to the formation of pulmonary edema. The proces of hydrolysis was thought to be the reason for the latent period between the injury and the onset of symptoms. The ability of the lungs to secrete lipase was demonstrated in several species (1,25,38,70,79).

Studies on the chemical composition of fat obtained from human long bones revealed that the fatty acids in this neutral fat were predominantly (65 % to 80 %) unsaturated (63). Oleic acid (60-70 %) and to a minor extent linoleic acid (6-10 %) were the most important fatty acids of human fat.

Activation of clotting cascade and complement system

Peltier's hypothesis is still subscribed by several investigators, but one need

not implicate this conversion to free fatty acids to explain the observed pulmonary failure (26). Disturbances in coagulation could also be important (4,9,46,68). Neutral fats and tissue thromboplastin released from the fracture site may activate the complement system (33), the extrinsic clotting cascade (84) and platelet aggregation. Accumulation of fat droplets, platelets, erythrocytes, leucocytes and fibrine may develop (26,59), probably stimulated by an inadequate functioning fibrinolytic enzyme system (67). Subsequently, the aggregations of fats, platelets, fibrine and leucocytes in combination with an activated complement system (30,88) and the release of numerous vasoactive substances or mediators may lead to membrane deformation and to an increase in pulmonary vascular permeability (26). In the last two decades it has become apparent that embolic fat and other elements could merely be a catalyst in a complicated chain of events leading to a final common pathway of increased pulmonary vascular permeability in response to many forms of systemic injury.

Oleic acid model

Historical

Since the first case of traumatic fat embolism was clinically recognized and published (7), experiments with animals have been performed to elucidate its pathogenesis (77). According to our knowledge, Jirka and Scuderi (1936) were the first to compare the effect of neutral fat (triolein) and a fatty acid (oleic acid) injection in dogs. They found oleic acid a far more toxic substance, indicated by a much lower minimal lethal dose (40). Later, the toxic potency of oleic acid was confirmed by many authors (31,39,42,45,55,61,70).

In 1968, Ashbaugh and Uzawa (2) presented an experimental model of fat embolism in spontaneous breathing dogs. They injected a mixture of free fatty acids. In this dog model clinical and pathological features of the respiratory distress of fat embolism were simulated. After the administration of 0.075 ml/kg oleic acid in one single injection, the dogs developed tachypnea and became progressively hypoxemic. Most of them died between the second and third hour after administration. Also higher doses (0.1 ml/kg and 0.5 ml/kg) were tested, which immediately caused death in all animals.

After this study oleic acid has been used by many authors in different doses and in different animals to perform studies on experimental fat embolism. Most of these studies were performed during mechanical ventilation. Mortality was up to 30 % (43,93).

In pigs three studies with oleic acid were performed (28,47,93). In these studies hypoxemia was moderate as can be concluded from P_{aO2} and P_{aO2}/F_{IO2}

(Table 6.1). Criteria for a severe hypoxemia are a P_{aO2} lower than 50 mmHg with an F_{IO2} greater than 0.6 (64), or a $P_{aO2}/F_{IO2} < 100$ (57).

Pathogenesis of lung injury after oleic acid administration

Toxic effect of oleic acid

In a meso-appendix preparation of the rat, in which perfusion experiments of the microcirculation were performed in vivo under visual control, it was demonstrated by de Ruiter (70) that the permeability of microvessels was increased by a decrease in pH as well as by a decrease in Ca^{2+} concentration of the perfusate. Perivascular edema and hemorrhages were seen. When oleic acid was added to the perfusate, a similar increase in permeability was found, which was potentiated by a lower Ca^{2+}-ion concentration in the perfusate. The author suggested that an interaction between oleic acid and Ca^{2+} of the intercellular junctions of the endothelial cells could be the reason for the increase in permeability.

Table 6.1. Oleic acid administration in pigs.

authors	dose	P_{aO2}	F_{IO2}	P_{aO2}/F_{IO2}
Halden et al.(28)	0.1 $ml.kg^{-1}$ i.v. in 30 min.	51	0.21	244
Zetterström et al. (93)	0.11 $ml.kg^{-1}$ i.v. in 30 min.	66	0.21	313
Kroese-Elliot and Olson(47)	0.022 $ml.kg^{-1}.h^{-1}$ * i.v. during 4 h.	50	0.21	238

* 20 $mg.kg^{-1}.h$-1, converted to $ml.kg^{-1}.h^{-1}$ using a specific gravity of 0.89.

King et al. (45) observed in vivo pulmonary microvessels in dogs after oleic acid administration via a thoracic window. They also noticed a toxic vasculitis with increased permeability, as described by de Ruiter.

In isolated dog lungs, depleted of blood components, massive edema developed after oleic acid administration (35). This edema was attributed either to a direct toxic effect of oleic acid on the endothelium or to the release of mediators by the endothelial cells after contact with the oleic acid. Other authors demonstrated that oleic acid inhibited the activity of the Ca^{2+} pump (65) and $(Na^{+}+K^{+})$-ATPase (48), leading to dysfunction of the cell membrane. Moreover, changes in lipid composition of lung microsomal membranes occurred (11).

Contributing factors

Although accumulation of platelets (86) and leukocytes (18,21,24,32,41,72) and deposition of fibrin (41,72) was seen in the lungs after oleic acid administration, these blood components do not appear to be essential for initation of this type of injury (19,35,43). This does not exclude, however, that these factors contribute to the injury in a later phase.

In dogs it was claimed that the oleic acid induced damage was partly due to production of oxygen radicals (89). However, treatment of oleic acid injected rats with oxygen radical inhibitors like catalase, superoxide dismutase, or dimethyl sulfoxide failed to inhibit lung permeability changes induced in this model (21).

Ball et al. (3) suggested some involvement of sulfidopeptide leukotrienes (LT) in oleic acid induced pulmonary injury in rats. They found an increase in LTC_4/D_4 and LTB_4 in broncho-alveolar lavage fluid after oleic acid administration. In pigs with oleic acid lung injury, these results could not be confirmed (47). Moreover the administration of an $LTD_4/LTDE_4$ receptor antagonist LY171883 failed to provide protection against the oleic acid induced cardiopulmonary changes. Therefore, the involvement of leukotrienes in oleic acid induced lung injury remains doubtful.

In dogs prostaglandin inhibition by meclofenamate enhanced blood oxygenation after oleic acid administration (73), implying a possible influence of prostaglandines.

Another factor that might contribute to the development of lung damage after oleic acid is mastcell degranulation leading to histamin release, as was demonstrated in the isolated guinea pig lung (80).

Albumin

Because albumine is capable to bind oleic acid (10,85) it has potentially a protective effect against the toxic properties of oleic acid. It was demonstrated

that albumine neutralized the toxic effects of oleic acid (16,70). Hofman and Ehrhart confirmed this result in an isolated lung lobe preparation of the dog (36), where albumine attenuated the deleterious effects of oleic acid.

The only prognostic index to indentify patients that developed fat embolism syndrome after trauma, was a significantly lowered albumine concentration (56).

Effects of oleic acid on gas exchange and lung mechanics

Oleic acid administration caused a decrease in arterial oxygen tension (P_{aO2}) (2,17,28,37,47,58) which appeared to be dependent on the dose (43). Some authors describe a large individual spread in the value of P_{aO2} after one single dose of oleic acid (75). In most studies the hypoxemia was moderate, P_{aO2}/F_{IO2} being greater than 100 and criteria of severe respiratory distress (57,64) were not fulfilled.

Several authors described a decrease in end-expiratory lung volume and total respiratory compliance after oleic acid administration (27,29). Surfactant function was altered severely (29). Baum et al. showed that oleic acid interfered with normal surfactant function both in vitro and in vivo (5,6). Administration of artificial surfactant (Exosurf) did not have any acute benificial effect on gas exchange and compliance (91).

Pulmonary hypertension

Oleic acid caused an elevation of pulmonary arterial pressure (2,8,43,74). In several studies the pressure-flow relationship was determined after oleic acid administration (8,49,50). It was suggested (50), that vasoconstriction is an important reason for the observed pulmonary hypertension.

PEEP in the oleic acid model

Many studies with positive end-expiratory pressure (PEEP) were performed in the oleic acid model. PEEP had a benificial effect on P_{aO2} in oleic acid lung injury (14,15,20,90), as a result of an increase of areas of adequately ventilated and perfused units (20). However, PEEP had a negative effect on cardiac output in this model (12,15,54,71,83). Thus, gas transport is positively influenced by PEEP via the improvement of arterial oxygen tension, but negatively via cardiac output. The level of PEEP where oxygen delivery, i.e. the product of cardiac output and oxygen content, is maximal is called "best PEEP" (87). Murray et al. (58) found this maximum after oleic acid administration at zero end-expiratory pressure. In their study, respiratory distress was hardly present, P_{aO2} was about 110 mmHg at an F_{IO2} of 0.5, implying a normal oxygen saturation.

Objectives

In many studies mentioned above, only a mild respiratory distress was obtained, $P_{aO2}/F_{IO2} > 100$. Many different doses were given, varying from 0.06 ml/kg to 0.5 ml/kg. Oleic acid was administered either as a single bolus or as a continuous infusion. A large individual spread in P_{aO2} was seen after a single infusion of 0.06 ml/kg. In some studies mortality was high, up to 30%.

We aimed primarily at a model of severe respiratory distress in pigs, caused by oleic acid administration, fulfilling the following conditions:

-criteria of respiratory distress according to Petty (64) and Murray et al.(57),
-a comparable degree of hypoxemia in all animals,
-a low mortality, and
-a stable distress for several hours to get the possibility of studying effects of different interventions.

We aimed further at an extensive physiological and morphological description of the model, including morphometry of the pulmonary muscular arteries, which then could serve as a control study for interventions.

Next we studied PEEP in the model to evaluate indicators of "best PEEP".

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CHAPTER VII

A STABLE MODEL OF RESPIRATORY DISTRESS BY SMALL INJECTIONS OF OLEIC ACID IN PIGS

Oleic acid has often been used to induce an experimental model of respiratory distress in animals (4,8,12,15,29,30,33,35,45,46,50). In these studies oleic acid was given either as a single bolus or as a continuous infusion. The amount was different between the different studies. Mortality was up to 30% (30,50). In most studies, respiratory distress criteria according to Petty (40) and Murray (39) were not fulfilled and interventions were studied without a proper description of the model.

We aimed at a model of an early stage of respiratory distress in pigs that fulfilled the clinical criteria of the adult respiratory distress syndrome (39,40), in which both gas exchange and hemodynamic variables were stable for several hours. Such a model can be used for studies on basic mechanisms and therapeutic interventions in early respiratory distress. We induced respiratory distress by multiple small injections of oleic acid.

METHODS

Surgical procedures and ventilatory conditions

Eight Yorkshire pigs (9.1 $\pm$ 0.7 kg) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 $mg.kg^{-1}$) and placed in supine position on a thermo-controlled operation table to maintain body temperature. Anesthesia was maintained by a continuous infusion of pentobarbital sodium (8.5 $mg.kg^{-1}.h^{-1}$). After tracheostomy the pigs were connected to a volume controlled ventilator.

A polythene single lumen catheter was inserted through the right common carotid artery into the aortic arch for measuring arterial blood pressure (P_{ao}) and sampling of blood. Three catheters were inserted via the right external jugular vein: 1) a Swan-Ganz catheter into the left pulmonary artery to monitor pulmonary arterial pressure (P_{pa}) and pulmonary blood temperature and to sample mixed venous blood; 2) a double walled catheter into the right atrium for injection of saline at room temperature during the thermodilution procedures, and 3) a four lumen catheter into the superior vena cava to measure central venous pressure (P_{cv}) and to infuse fluids and anesthetics. All catheters for measuring blood pressures were continuously flushed at a flow rate of 3 $ml.h^{-1}$ with normal saline containing a low dose of heparine (10 I.U. per ml infusion fluid) to avoid

clotting in the catheters. A catheter was put into the urinary bladder to avoid retention of urine.

After the surgical procedures, tubocurarine was given at a rate of 0.2 $mg.kg^{-1}.h^{-1}$ to suppress spontaneous breathing. Then, mechanical ventilation was changed to a computer controlled ventilator, developed in our laboratory (27), containing two separate, in parallel functioning bellows. One bellows served for continuous ventilation throughout the experiments, the other for ventilation during determinations of lung volume. Ventilation was set on 10 breaths per min, a tidal volume (V_T) adjusted to a P_{aCO2} of 38-42 mmHg during baseline, an inspiratory to expiratory ratio of 2:3, an inspiratory fraction of oxygen (F_{IO2}) of 0.6 in nitrogen (N_2) and a positive end expiratory pressure (PEEP) of 2 cmH_2O. All settings were kept constant throughout the experiments.

Measured and estimated data.

Gas exchange, acid-base indices and hemoglobin

Oxygen and carbon dioxide tensions and acid-base indices of blood were determined with use of an automatic blood gas analyzer (Radiometer ABL3). Hemoglobin (Hb) concentration and O_2 saturation were determined with use of an oxymeter (Radiometer OSM2). Values of arterial oxygen saturation (S_{aO2}) measured in diluted blood, after dextran, were corrected based on the data of the manual of the OSM2. Inspiratory and mixed expiratory gases, including helium (He), were analyzed by a mass spectrometer (Perkin-Elmer, MGA 1100).

Arterial oxygen content (C_{aO2}) and venous admixture (Q'_s/Q'_t) in percentage of total pulmonary blood flow (Q'_t) were calculated according to (6):

$$C_{aO2} = (1.39\ Hb\ S_{aO2})/100 + 0.0031\ P_{aO2}$$

$$Q'_s/Q'_t = 100\ (C_{cO2}-C_{aO2})/(C_{cO2}-C_{\bar{v}O2})$$

where -1.39 is oxygen binding capacity in ml O_2 per g Hb,
-0.0031 is solubility of oxygen in blood in ml O_2 per 100 ml per mmHg,
-S_{aO2} is arterial oxygen saturation in %,
-P_{aO2} is arterial oxygen tension in mmHg,
-C_{cO2} is pulmonary end-capillary oxygen content in ml O_2 per 100 ml blood, and
-$C_{\bar{v}O2}$ is mixed venous oxygen content in the pulmonary artery in ml O_2 per 100 ml blood.

The saturation of the pulmonary end-capillary blood was derived from the oxygen saturation curve of pig blood and the alveolar oxygen tension (P_{AO2}), as a substitute for the pulmonary end-capillary tension. The parameters in the equation of the human oxygen saturation curve (31) were fitted for the pig's oxygen saturation curve, based on pig blood data from other experiments in our laboratory. This oxygen saturation curve was in

accordance with the curve described by Bartels and Harms (5).

Alveolar P_{O2} was derived from (3):

$$P_{AO2} = P_{IO2} - P_{ACO2} [F_{IO2} + (1-F_{IO2})/R]$$

where P_{IO2} and F_{IO2} are the inspiratory oxygen tension and fraction. P_{ACO2} is the alveolar carbon dioxide tension, which we assumed to be equal to P_{aCO2}. R is the respiratory quotient.

Physiological dead space (V_D/V_T) in percentage was obtained from the equation (16):

$$V_D/V_T = 100 (P_{aCO2} - P_{\bar{E}CO2})/P_{aCO2}$$

where $P_{\bar{E}CO2}$ is the mixed expiratory carbon dioxide tension.

Pulmonary data

End-expiratory lung volume

End-expiratory lung volume was estimated with use of an open He wash-in and wash-out technique. At the end of an expiration, mechanical ventilation was switched to the in parallel functioning bellows which was filled with an inspiratory gas mixture containing 4-5% He. During the next 90 seconds the lungs were ventilated with the He gas mixture to wash in the He. Then, ventilation was resumed with the O_2-N_2 mixture to perform the He wash-out. He fractions were continuously measured by a mass spectrometer during the whole procedure. During the wash-in period the end-expiratory lung volume (V_{EE}) was estimated at the end of each expiration from a) the inspired amount of He since the start of the wash-in, b) the expired amount of He since the start of the wash-in, and c) the He fraction in the lung after the last expiration, which we assumed to be equal to the measured fraction at the end of that last expiration, $F_{EE,He}$.

V_{EE} was calculated according to:

$$V_{EE} = (\int V'.F_{I,He}.dt - \int V'.F_{E,He}.dt)/F_{EE,He}$$

where V' is the airflow rate, $F_{I,He}$ is the inspiratory fraction of He, $F_{E,He}$ is the expiratory fraction of He. The values of V_{EE} at the end of the 90 s period are presented.

Total respiratory compliance

The compliance of lungs and thorax (C_{rs}) was estimated with use of an inspiratory pause method (10). Tracheal pressure (P_T) was measured in the cannula with a fluid filled catheter, provided with side holes, and connected to a Baxter disposable pressure transducer type Uniflow. Inspiratory volumes of 6, 12 and 18 ml per kg body weight were injected at intervals of 2 min during normal mechanical ventilation. Each insufflation was followed by a pause of 3 s. During these pauses tracheal pressure and thoracic volume decreased gradually. The volume change during a pause was recorded

with use of a mercury cord, which was fixed around the thorax at 5 cm cranial from the sternal xyphoid. Volume and pressure at the end of the inspiratory pause served as the data for the compliance estimation. A third degree polynomial pressure-volume curve was fitted through these data and through end-expiratory pressure and volume. This P-V curve implied an approximately linear part between the volumes 4 and 8 $ml.kg^{-1}$. The slope of this part was used as the compliance estimate (25). Throughout the experiments changes in thoracic volume were monitored with the mercury cord. C_{rs} estimates before and after the oleic acid injections were compared at the same thoracic volume level. Assuming a constant chest wall compliance (46), the changes in C_{rs} indicated changes in lung compliance.

The mercury cord was calibrated with the three insufflated volumes mentioned above, by recording its resistance change during insufflation. The stability of the mercury cord was tested during a period of ten hours by alternate stretch and release to an amount corresponding with ventilation and at a rate of 10 per minute. We did not observe any change in zero level and gain.

Hemodynamic data and oxygen delivery

P_{ao}, P_{pa} and P_{cv} were measured continuously with use of Baxter disposable pressure transducers, type Uniflow. Pressure values were referred to ambient air pressure and to a zero level at the height of the manubrium, and presented as mean values over a ventilatory cycle. Transducers were calibrated by application of pressure to this reference level under guidance of a mercury manometer.

Cardiac output (Q'_t) was determined by the thermodilution technique. The average of four determinations equally spread over the ventilatory cycle was used as the estimate of mean cardiac output (26).

Oxygen delivery (D_{O2}) was calculated according to:

$$D_{O2} = C_{aO2} \times Q'_t$$

Data acquisition

Throughout the experiments all blood pressures, ECG, P_T, V' and the resistance of the mercury cord were continuously recorded on a Gould recorder type RS 3800. During the estimations of Q'_t all other hemodynamic signals were sampled (250 Hz) on line by a computer. The sampling period was 18 s, i.e. 3 ventilatory cycles. P_T, V' and the mercury cord signal were sampled for 9 s at 100 Hz during the estimations of C_{rs}. During the open wash-in and wash-out procedures the in- and expiratory gases were sampled by a computer at 50 Hz. All signals were also stored on a Racal thermionic store 14, electromagnetic tape recorder.

Experimental procedures and observations

Protocol of the experiment.

After the surgical procedures and the change to the computer controlled ventilator, a stabilisation period of at least half an hour followed. Then, baseline observations were done in a period of one hour. Q'_t and gas exchange variables were determined twice in this hour. V_{EE}, C_{rs} and chest X-rays were obtained once during the baseline period.

After the baseline measurements 10 ml isotonic dextran-40 per kg body weight was given in half an hour via a lumen of the Swan-Ganz catheter and the double walled injection catheter. Immediately after the dextran infusion the circulatory and gas exchange variables were measured again. Then, as a control on the solvent of the oleic acid, 16 injections of 0.1 ml 96 % alcohol-saline solution (1:1) at 90 s intervals were given into the right atrium via the Swan-Ganz catheter. Immediately after these injections the measurements were repeated, which was about 25 min after the infusion of dextran.

After the control observations on alcohol, commercial oleic acid (Unichema International, specific gravity 0.89), dissolved 1:1 in 96 % alcohol, was given through the Swan-Ganz catheter into the right atrium. Injections of 0.1 ml oleic acid were given at intervals of 90 s until a stable P_{aO2} below 60 mmHg was achieved. Occasionally, we lengthened this interval and decreased the amount of oleic acid to 0.05 ml when a preceding injection caused a too large effect on the circulation. After each injection the catheter was flushed with 1 ml saline (40 °C). In pilot experiments we also applied larger amounts of oleic acid (0.2 to 1 ml), which immediately caused cardiovascular shock and death. In other pilot experiments on the series of 0.1 ml injections, P_{aO2} hardly changed until the 10th injection. Therefore, in our definite study P_{aO2} was measured after the first 10 injections, next after each four or two injections and at the end after each single injection. We continued oleic acid administration until the P_{aO2} was below 60 mmHg.

The last oleic acid injection was taken as zero time of the observation period during respiratory distress. All measurements of gas exchange and cardiac output were performed at 15, 30, 45, 60, 90, 120 min and then every hour. All continuously monitored signals were also sampled at these time intervals. Estimations of V_{EE} and C_{rs} were done at 90, 120 and 180 min after the last injection of oleic acid. In some experiments estimations of V_{EE} and C_{rs} failed because of formation of bloody froth in the tracheal cannula or due to technical reasons. At the end of the experiments chest X-rays were obtained again.

Lung weight and morphology

At the end of the experiments, the animals were killed with pentobarbital sodium (0.07 $g.kg^{-1}$). Immediately after death the lungs were fixed by instillation of formalin through the trachea. After ligation of blood vessels, heart and lungs were removed and weighed. The amount of formalin was subtracted from the weight of the heart and lungs. Blocks of tissue were taken from apical and diaphragmatic lobes on either side. Slides were cut from these blocks and stained with hematoxylin and eosin and with an elastic-van Gieson stain for histologic examination.

In some experiments a small block was taken from the ventral part of the left diaphragmatic lobe, for electron microscopic investigation. These small blocks were fixed in a glutaraldehyde solution. After post fixation with osmium, the blocks were dehydrated with acetone, embedded in LX112 and stained with uranyl acetate and lead citrate.

Criteria of respiratory distress

A criterion of respiratory distress, mentioned by Petty (40), is a P_{aO2} of 50 mmHg with an F_{IO2} of 0.6 at zero PEEP. This P_{aO2} value was used as a target during the oleic acid injections and the period after these injections. Murray (39) suggested an expanded definition of respiratory distress using a score system to characterize the presence and severity of the disease based on the following features: chest roentgenogram score, hypoxemia score (P_{aO2}/F_{IO2}), positive end-expiratory pressure score and respiratory system compliance score. To determine the score of C_{rs} in our pigs, we recalculated Murray's values per kg body weight, assuming an average weight of 70 kg in his patients. This score system was implied in the evaluation of our model.

Statistical analysis

The results were analyzed using standard repeated measures analysis of variance (SPSS-Manova) or student t-tests for paired and unpaired samples. P-values ≤ 0.05 were accepted as statistically significant. Data are presented as mean values $\pm$ 1 sd.

RESULTS

Control observations

Dextran infusion

The effects of dextran infusion are presented in Table 7.1. The main effects are a decrease in Hb concentration and an increase in cardiac output, blood pressures and oxygen delivery.

Table 7.1. Control data and data after oleic acid administration.

	baseline n=6	dextran n=6	alcohol/NaCl n=6	oleic acid 60 min n=6
P_{aO2}(mmHg)	292 ± 26.8	294 ± 30.9	315 ± 33.7	54.9 ± 6.1 §
P_{aCO2}(mmHg)	40.9 ± 3.0	40.7 ± 3.8	42.9 ± 4.9	58.3 ± 9.2 §
S_{aO2}(%)	99.2 ± 1.1	99.0 ± 1.6	98.9 ± 1.6	72.0 ± 7.5 §
Q'_s/Q'_t(%)	7.2 ± 2.0	11.2 ± 2.7 **	-	42.8 ± 6.0 (n=5) ##
V_D/V_T (%)	33.8 ± 10.8	28.0 ± 8.2	-	58.5 ± 3.1 (n=5) ##
Hb(mmol.l^{-1})	6.5 ± 0.5	5.0 ± 0.4 **	5.3 ± 0.5 **##	7.6 ± 1.2 §
Q'_t(ml.s^{-1}.kg^{-1})	2.1 ± 0.3	3.1 ± 0.4 **	2.8 ± 0.4 ** #	1.4 ± 0.1 §
P_{ao}(mmHg)	99.7 ± 10.9	112 ± 14.7 *	112 ± 13.1 *	97.8 ± 13.9
P_{pa}(mmHg)	14.7 ± 2.4	18.7 ± 1.7 **	18.2 ± 2.4 **	37.6 ± 2.0 §
P_{cv}(mmHg)	1.5 ± 0.8	3.4 ± 1.2 **	2.6 ± 1.2 *##	3.5 ± 1.4 §
D_{O2}(ml.s^{-1}.kg^{-1})	32.7 ± 4.4	37.5 ± 6.7 *	35.3 ± 5.8	17.9 ± 1.2 §
$P_{T,p}$(cmH_20)	21.9 ± 5.3	21.5 ± 5.0	22.1 ± 6.3	36.2 ± 3.1 §
pH	7.46 ± 0.04	7.47 ± 0.04	7.45 ± 0.04	7.32 ± 0.06 §
HCO_3^-(mmol.l^{-1})	28.8 ± 1.7	29.1 ± 1.8	28.7 ± 1.5	25.6 ± 2.1 §

Values are mean ± sd; Q'_s/Q'_t and V_D/V_T at 45 min after oleic acid; n=5, due to technical reasons; P_{aO2}, arterial P_{O2}; P_{aCO2}, arterial P_{CO2}; S_{aO2}, arterial oxygen saturation; Q'_s/Q'_t, venous admixture; V_D/V_T, physiological dead space; Hb, hemoglobin concentration; Q'_t, cardiac output; P_{ao}, arterial pressure; P_{pa}, pulmonary arterial pressure; P_{cv}, central venous pressure; D_{O2}, oxygen delivery; $P_{T,p}$, peak tracheal pressure; pH, arterial pH; HCO_3^-, standard bicarbonate concentration. * $p \leq 0.05$ compared to baseline, ** $p < 0.01$; # $p \leq 0.05$ compared to dextran, ## $p < 0.01$; § $p < 0.01$ compared to alcohol-NaCl.

Alcohol-saline injections

We did not observe any change in the continuously measured variables after single injections of the alcohol-saline solutions. After the series of injections Hb concentration was increased slightly and cardiac output and central venous pressure were decreased (Table 7.1). All other variables remained constant.

Oleic acid injections

The administration of oleic acid injections of 0.1 ml had extensive hemodynamic effects, as demonstrated in an individual example (Fig. 7.1).

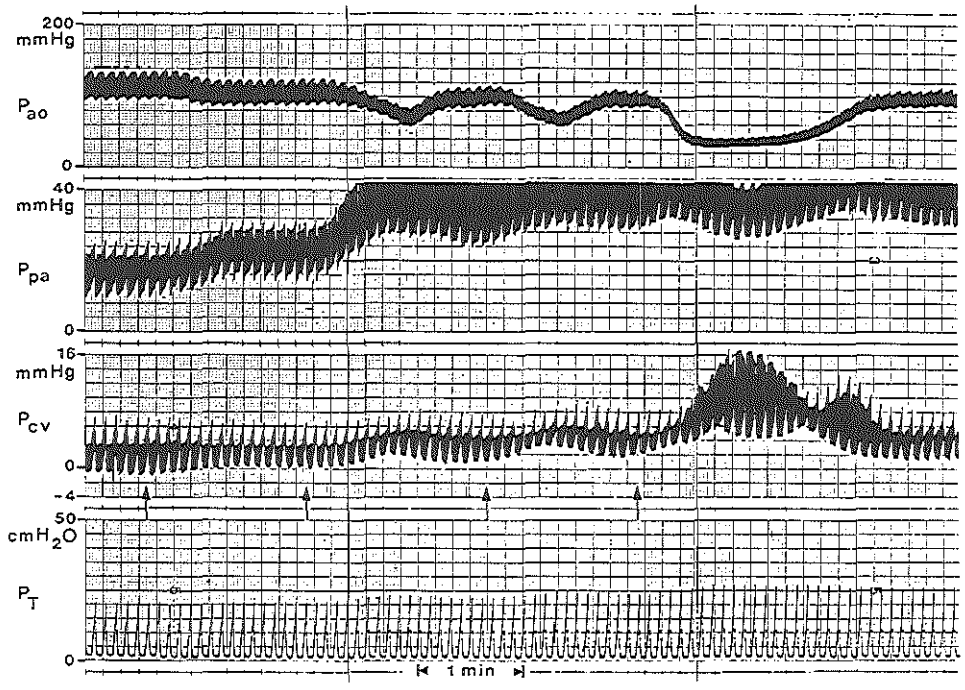


Fig. 7.1. Hemodynamic effects of oleic acid injection.
Four out of eight signals are shown. ECG, air flow (V'), the mercury cord signal of thoracic circumference and blood temperature were left out. P_{ao}: arterial pressure, P_{pa}: pulmonary arterial pressure, P_{cv}: central venous pressure, P_T: tracheal pressure. Each arrow indicates an injection of 0.1 ml oleic acid. The time interval after the second injection was lengthened because of the severe hemodynamic reactions.

These effects varied in severity between injections within an experiment as well as between animals. The injections caused an immediate rise of P_{pa} and P_{cv}, and a decrease in P_{ao} and arterial pulse pressure.

When severe circulatory reactions were observed, resulting in a high P_{cv} and a low P_{ao}, the interval between the injections was lengthened until these variables were partly recovered and stable again. When an injection caused a mean P_{ao} < 40 mmHg, a critical level below which the coronary flow is dependent on pressure (7), we diminished the next injection to half the amount (0.05 ml).

One of the eight animals died after the 12th injection, although the last eight injections were given with half the dose. P_{aO2} was 271 mmHg at that time. After the oleic acid injections in the remaining seven animals a stable hypoxemia was established in all but one animal (Fig. 7.2). In this one animal P_{aO2} recovered after 30 min (70 mmHg), reaching a maximum of 95 mmHg after two hours. Therefore, the data of this animal was eliminated from the study.

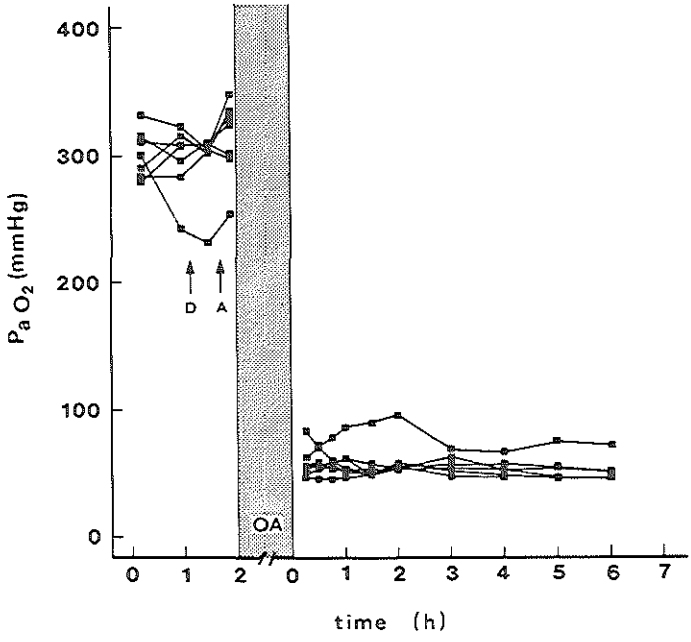


Fig. 7.2. Individual responses of P_{aO2}.
Baseline observations were done in the first hour. D: Start of dextran infusion (30 min), A: Start of alcohol-saline injections (25 min), OA: Oleic acid injections, h: hours. The interrupted X-axis indicates the time difference of oleic acid administration in the different animals (n=7). Zero time at the end of the shaded area corresponds with the moment of the last oleic acid injection.

Table 7.2. Oleic acid administration (n=6).

	mean	sd	range
Number of injections	22	11	12 - 42
Injection time (min)	75	38	39 - 141
Total amount ($ml.kg^{-1}$)	0.12	0.07	0.06 - 0.25

In another animal, where P_{aO2} was 80 mmHg at the end of the oleic acid injections, bloody froth appeared in the tracheal cannula before the target P_{aO2} was reached. We stopped the oleic acid injections. Nevertheless P_{aO2} decreased to a value below 60 mmHg before the end of the first hour and remained stable throughout the experiment. This animal was kept in the group. Thus, in six out of eight animals a stable distress was obtained.

The number of oleic acid injections, the time to carry out these injections and the total dose of oleic acid are given in Table 7.2. This data indicates a large individual spread in the amount of oleic acid necessary to cause a respiratory distress.

Characteristics of the oleic acid model

The effects of oleic acid on gas exchange and hemodynamic variables one hour after the last injection are presented in Table 7.1. A few measurements failed by technical reasons, as indicated in the table.

During the distress period two animals died after 3.5 and 4 hours because of a circulatory shock. The standard bicarbonate (HCO_3^-) concentrations were 22 and 18 $mmol.l^{-1}$ respectively in those animals. One experiment ended after 5 hours because of technical reasons, whereas the animal was in a stable condition at that time. The remaining three animals were studied for 6 hours.

We restricted the presentation of averaged data and the statistical testing (n=5) to a period of 4 hours after the last oleic acid injection. For Q'_s/Q'_t and V_D/V_T statistical testing was limited to the first two hours because of missing values after this period.

Gas exchange, acid-base indices and hemoglobin

P_{aO2} was decreased profoundly by the series of oleic acid injections (Table 7.1) and remained stable during the distress period (Fig. 7.3.a). After this period of 4 h, individual values were similar (Fig. 7.2). The decreased S_{aO2} and increased Q'_s/Q'_t did not change significantly during the distress period (Figs. 7.3.b and 7.3.c). P_{aCO2} was increased and pH and HCO_3^- concentration were decreased after the oleic acid administration. These variables did not change significantly during the distress period (Figs. 7.3.d-7.3.f). V_D/V_T was doubled after the oleic acid administration and remained at this level (Fig. 7.3.g).

Hb concentration, which was reduced after volume expansion with dextran, increased during the period of oleic acid injections to a value above its baseline level (Table 7.1). In the distress period Hb concentration remained approximately the same (Fig. 7.4).

Pulmonary data

P_T at peak insufflation ($P_{T,p}$) was increased after oleic acid administration and rose steadily during the 4 h distress period to 40.7 $\pm$ 6.8 cm H_2O ($p=0.02$, Fig. 7.5). In the four animals that could be studied beyond this period of 4 h, individual values showed a similar positive trend until the end of the experiments.

V_{EE} was decreased from 21.0 $\pm$ 2.6 $ml.kg^{-1}$ ($n=6$) in baseline to 10.9 $\pm$ 2.9 $ml.kg^{-1}$ ($p<0.05$, $n=4$) at 90 min after the last oleic acid injection (Fig. 7.6). Estimations at 120 min ($n=3$) and 180 min ($n=3$) were similar to the values at 90 min after oleic acid administration.

C_{rs} was decreased from 1.80 $\pm$ 0.3 $ml.cmH_2O^{-1}.kg^{-1}$ in baseline to 0.85 $\pm$ 0.3 $ml.cmH_2O^{-1}.kg^{-1}$ ($p<0.05$, $n=5$) at 90 min after the last oleic acid injection (Fig. 7.7). Individual values of C_{rs} in 3 animals at 120 min and 180 min were similar to their values at 90 min.

Hemodynamic data and oxygen delivery

Q'_t was significantly decreased after the oleic acid injections to a value below its baseline value in spite of the dextran administration (Table 7.1). Subsequently, Q'_t remained stable during the distress period (Fig. 7.8.a). P_{ao} was not different from baseline after the oleic acid injections and remained at this level (Fig. 7.8.b). P_{pa} and P_{cv} increased during the series of oleic acid injections and did not change significantly in the distress period (Figs. 7.8.c and 7.8.d).

D_{O2} was reduced to about 50 % after oleic acid and was stable throughout the distress period (Fig. 7.8.e).

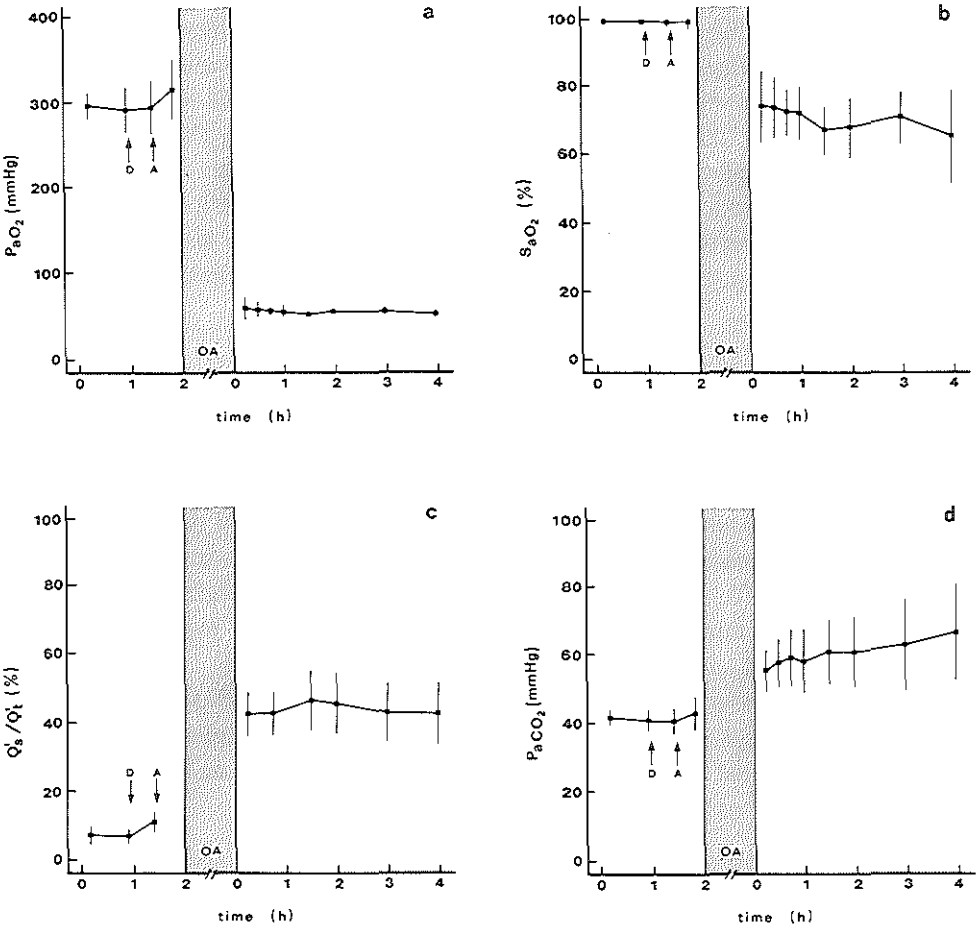
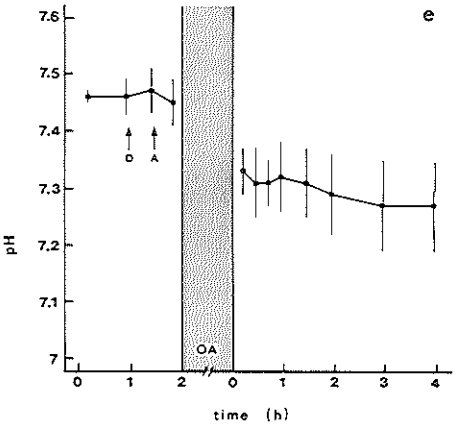
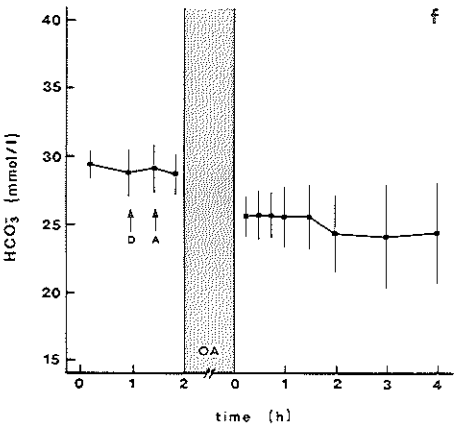
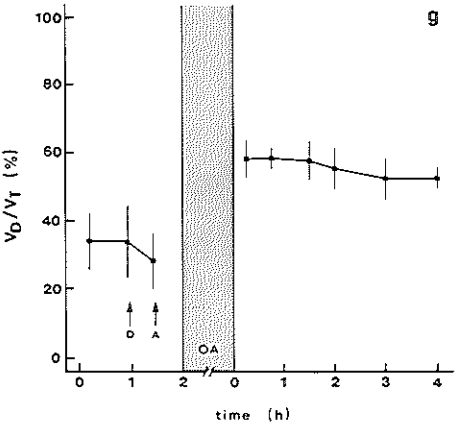


Fig. 7.3. Effects of oleic acid on gas exchange variables.
Time scale as in Fig. 7.2, limited to 4 hours after oleic acid administration. Abbreviations as in Fig. 2. Mean values of 6 animals; at t=4 h, n=5. For venous admixture and physiological dead space mean values of 5 animals; at t=3 h, n=4 and at t=4 h, n=3. Vertical bars: sd. a. arterial oxygen tension, b. arterial oxygen saturation, c. venous admixture, d. arterial carbon dioxide tension, e. pH, f. HCO_3^- concentration, g. physiological dead space. Venous admixture and physiological dead space were not determined immediately after the alcohol-saline injections.


e
pH
7.6
7.5
7.4
7.3
7.2
7.1
7
D
A
OA
0 1 2 0 1 2 3 4
time (h)


f
HCO3 (mmol/l)
40
35
30
25
20
15
D
A
OA
0 1 2 0 1 2 3 4
time (h)


g
VD/VT (%)
100
80
60
40
20
0
D
A
OA
0 1 2 0 1 2 3 4
time (h)

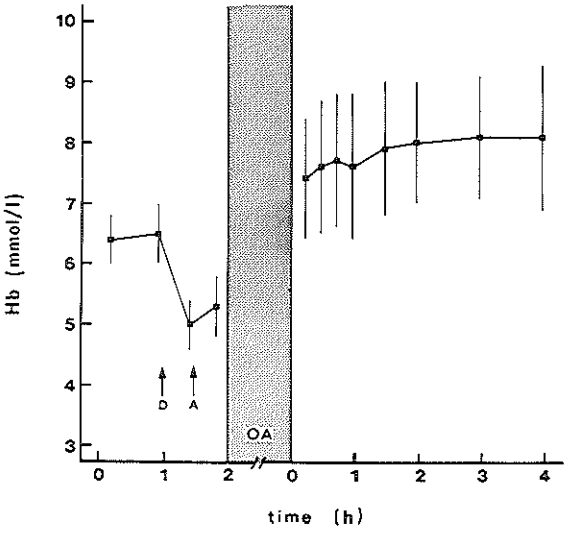


Fig. 7.4. Hemoglobine concentration.
Abbreviations and time scale as in Fig. 7.3. Mean values of 6 animals; at t=4 h, n=5. Vertical bars: sd.

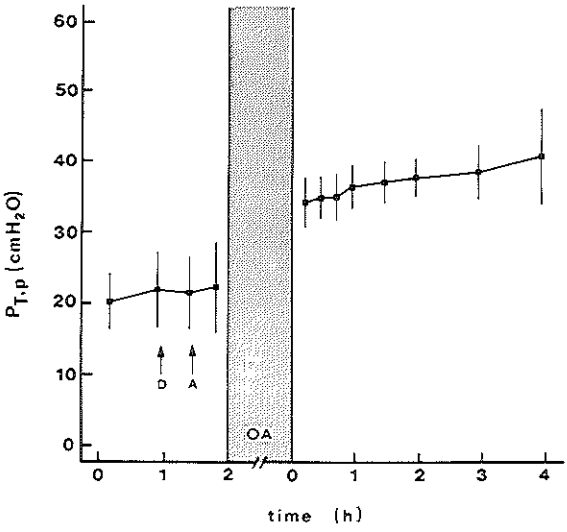


Fig. 7.5. Peak tracheal pressure.
Abbreviations, vertical bars and time scale as in Fig. 7.3. Mean values of 6 animals; at t=4 h, n=5.

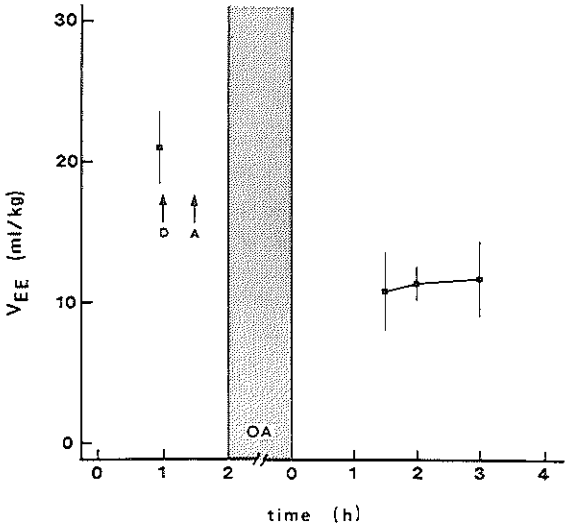


Fig. 7.6. End-expiratory lung volume.
Time scale and vertical bars as in Fig. 7.3. n=6 before oleic acid; at 90 min, n=4; at 2 and 3 h, n=3.

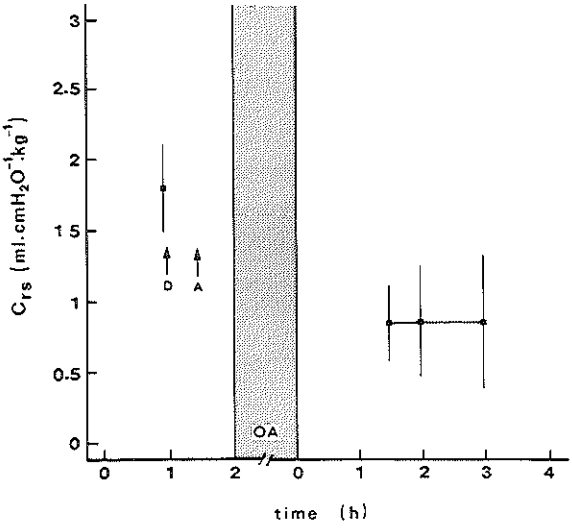


Fig. 7.7. Total respiratory compliance.
Time scale and vertical bars as in Fig. 7.3. n=6 before oleic acid; at 90 min, n=5; at 2 and 3 h, n=3.

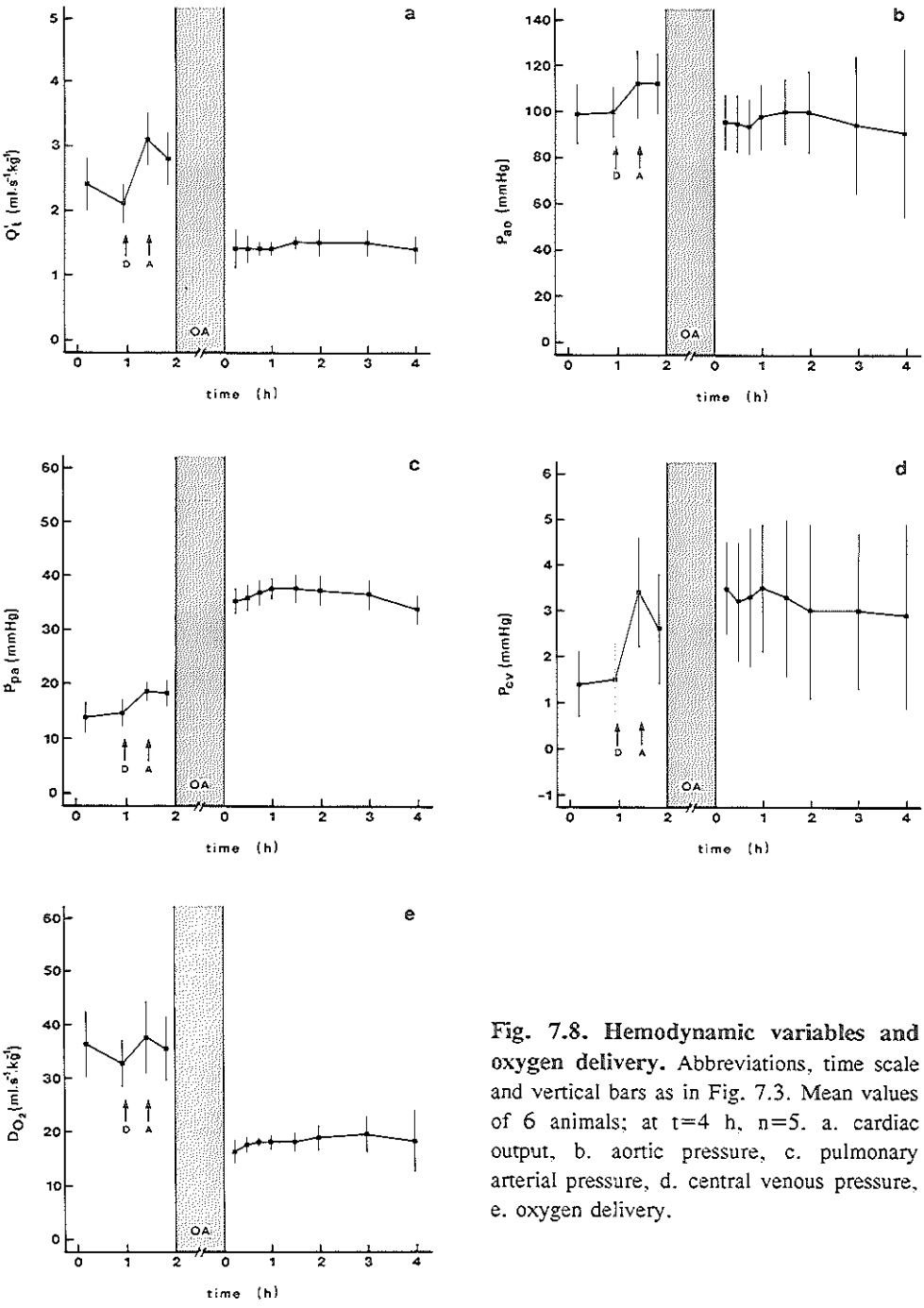


Fig. 7.8. Hemodynamic variables and oxygen delivery. Abbreviations, time scale and vertical bars as in Fig. 7.3. Mean values of 6 animals; at t=4 h, n=5. a. cardiac output, b. aortic pressure, c. pulmonary arterial pressure, d. central venous pressure, e. oxygen delivery.

Lung weight and morphology

The weight of lungs and heart in a control group of six animals, obtained from another study at our laboratory, was 20.7 ± 2.2 $g.kg^{-1}$. The weight in the oleic acid group was 33.2 ± 4.1 $g.kg^{-1}$, which was significantly larger ($p<0.01$).

Histologic examination revealed an extensive interstitial edema and an acute bronchopneumonia in all lobes (Fig. 7.9), with some interstitial pneumonia. The lesions varied from mild and focal to severe and diffuse. There was extensive vasculitis in both arteries and veins (Fig. 7.10), and in some arteries, fibrinoid necrosis and fibrine thrombi were found. There were signs of congestion in the inflammated areas. We never observed hyaline membranes. Muscular pulmonary arteries were constricted, though there was some variation in severity.

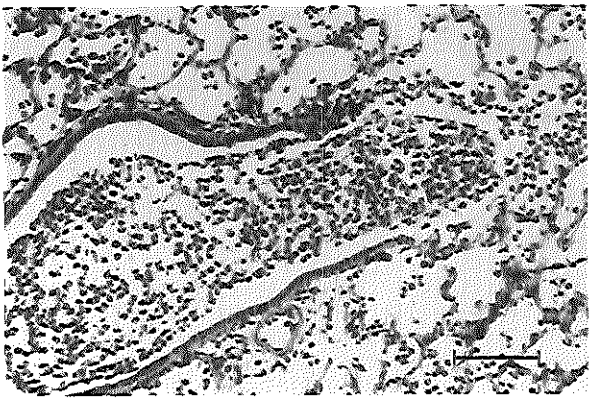


Fig. 7.9. Acute bronchopneumonia. Hematoxylin and eosin, x150. Bar, 100 μm.

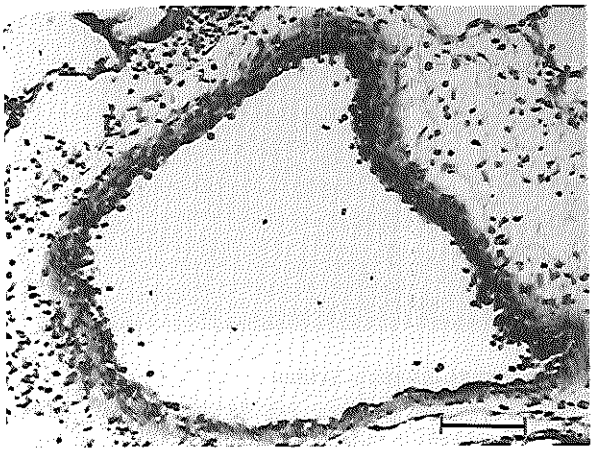


Fig. 7.10. Pulmonary vein. There is prominent vasculitis. Hematoxylin and eosin, x150. Bar, 100 μm.

Electron microscopy revealed swelling of endothelial cells. These cells protruded into the vascular lumen and showed lysosomes and vacuoles as signs of degeneration (Fig. 7.11). There was also some swelling of the internal elastica lamina. The pneumocytes II had a normal appearance (Fig. 7.12).

Criteria of respiratory distress

The stable P_{aO2} below 60 mmHg in our model approximated the physiological criterion of P_{aO2} for the adult respiratory distress syndrome ($P_{aO2} < 50$ mmHg, $F_{IO2} \geq 0.6$, PEEP=0 cmH_2O), as defined by Petty (40). Evaluation of our oleic acid model with the score system described by Murray (39), revealed a moderate to severe respiratory distress (averaged score of all animals 2.4 $\pm$ 0.1). The chest x-rays showed alveolar edema in all lung lobes of the 6 animals (value=4), the hypoxemia score (P_{aO2}/F_{IO2}) was below 100 in all animals (value=4) and PEEP was below 5 cm H_2O (value=0). C_{rs} was between 0.57 and 0.84 $ml.cmH_2O^{-1}.kg^{-1}$ in 3 animals (value=2) and between 0.84 and 1.13 $ml.cmH_2O^{-1}.kg^{-1}$ (value=1) in the other 3 animals.

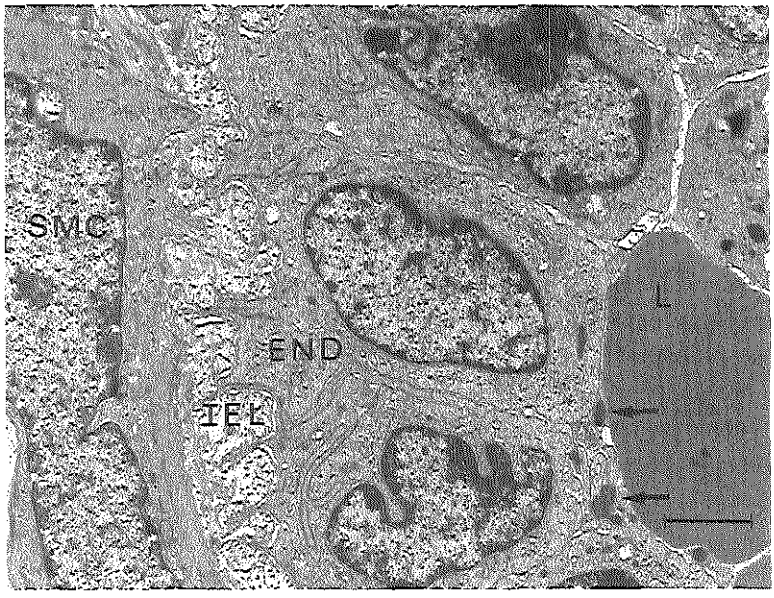


Fig. 7.11. Electron micrograph of muscular pulmonary artery.
There is swelling of endothelial cells (END), protruding in the vascular lumen. The internal elastic lamina (IEL) is markedly crenated. The cytoplasm of endothelial cells contains lysosomes (arrows). L, lumen containing erythrocytes; SMC, nucleus of smooth muscle cell; x4400. Bar, 1.5 μm.

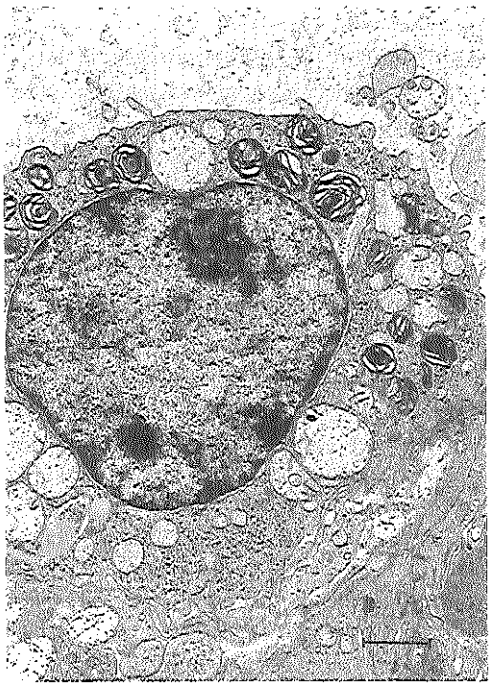


Fig. 7.12. Electron micrograph of type II pneumocyte in pig lung, after oleic acid administration. The lamellar bodies have a normal aspect; x7000, Bar 1 μm.

DISCUSSION

Control observations

Dextran infusion

Volume expansion was applied by several authors, but so far we could not find a standardized regime. It was done either before (22), or during and after (20), or only after the administration of oleic acid (17). To evaluate the effects of oleic acid, we avoided interventions in parallel with the oleic acid administration and infused dextran prior to the injections of oleic acid. The volume expansion increased cardiac output to 50 % above its baseline value. Q'_s/Q'_t increased slightly more, which was probably due to the increase in Q'_t (49). It has been suggested that a higher pulmonary blood flow might lead to a relative increase in perfusion of non-ventilated areas in the lungs (11).

The alcohol-saline injections

The series of alcohol-saline injections did not affect gas exchange and other pulmonary functions. We regard the slight increase in Hb and the decrease in Q'_t and P_{cv} to be an effect of counteracting neuro-humoral control mechanisms on the preceding volume expansion (2,18). Because the effects of alcohol were negligible, we attributed the changes in gas exchange and circulation after the oleic acid-alcohol injections solely to the oleic acid.

The total amount of alcohol used in our experiments was on average 0.2 $ml.kg^{-1}$. The maximal amount injected in one of the experiments was 0.3 $ml.kg^{-1}$. For a human adult this would be about 21 ml alcohol, corresponding with a consumption of one and a half glass of wine. Undoubtedly, alcohol was metabolized during application of the series of injections, leading to a lower effective dose than calculated. Alcohol needs to be given in a dose equal to 5 times our maximal dose to potentiate the anesthetic action of pentobarbital (42). We consider the amount of alcohol, used in our experiments, too small for such an effect.

The regime of oleic acid administration.

Davis and Dubos (13) demonstrated that the toxic effects of oleic acid could be neutralized by albumine. This was also found in an isolated perfused lung lobe, where albumine solution attenuated the deleterious effects of oleic acid (23). Under normal circumstances unsaturated fatty acids are almost completely bound by albumine (47). If all binding sites of albumine for fatty acids are occupied, the concentration of free fatty acids increases suddenly during further administration (47). Presumably, fatty acids have only a toxic effect on the alveolar-capillary membrane, leading to disturbances in gas exchange, if the concentration of the free fatty acids exceeds the binding capacity of albumine in blood. Such concentration can be regarded as a threshold. This threshold concentration seems to be individually determined, as in our experiments the amount of injections necessary to obtain a respiratory distress varied between 12 and 42. Such variation could depend on the amount of free binding sites for fatty acids.

Because the threshold concentration of a particular pig is not a priori known, a single bolus injection or a continuous infusion is less suitable to create a reproducible model in different animals. In such types of application an excessive amount of oleic acid could surpass the fatty acid binding capacity of albumine leading to severe pulmonary edema, cardiovascular problems and death of the animal. Some authors report a mortality of 25 and 30 % (30,50). The application of multiple small doses of oleic acid can be adapted to individual differences in

threshold concentration, avoiding death of the animals. In our study mortality was only one out of eight.

Kruse-Elliot and Olson (33) used a continuous infusion of oleic acid (20 $mg.kg^{-1}.h^{-1}$) in pigs during the whole experiment. They reported a hypoxemia after 1 h, i.e. P_{aO2} was about 50 mmHg with ventilation at room air, that remained constant for a period of four hours. However, in another group of pigs the mortality was 100 % within 2.5-3 hours during such a continuous infusion. The higher mortality in the second group could have been due to a lower binding capacity of albumine for free fatty acids. We applied on average 106 mg oleic acid per kg body weight in a period of about 75 min (Table 7.2) to establish a stable respiratory distress for 4 to 6 hours. Thus, it is not necessary to continue an infusion of oleic acid once a respiratory distress is established.

Because in one animal P_{aO2} started to recover about 30 min after the last oleic acid injection and in another P_{aO2} decreased a bit further, we recommend to wait for at least 30 min to observe stability before starting a study of some intervention. When recovery of P_{aO2} occurs, the experiment should be either eliminated from the study, or an additional small amount of oleic acid should be tried.

The distress model

Pathogenesis

The oleic acid induced lung injury is probably primarily caused by a direct toxic effect on the endothelial wall (24,28). A mechanism might be the potency of oleic acid to inhibit the activity of the Ca pump and $(Na^+ + K^+)$-ATPase (34, 41), leading to dysfunction of the cell membrane. Electron microscopical investigation in our study revealed signs of degeneration of the endothelium, like swelling and vacuolisation. These alterations were also mentioned by Derks and Jacobovitz-Derks (14). Mediators like leukotrienes, phospholipase A or cyclo-oxygenase metabolites and the increased number of leucocytes (14) and aggregated thrombocytes (48) seen in the lungs after oleic acid may contribute to the pathogenesis of this type of lung injury (24,30,33).

Gas exchange and acid base data

In our animals a moderate to severe respiratory distress, according to Murray's criteria (39), was induced. P_{aO2} decreased to a level of about 50 mmHg, close to one of Petty's physiological criteria of ARDS, and remained stable until the end of the experiments. Venous admixture was increased after oleic acid, indicating the presence of low ventilation-perfusion ratio's (20). The

increased P_{aCO2} indicated that the effective alveolar ventilation was decreased after oleic acid induced lung injury; we found a doubling of physiological dead space in our animals. The decrease in pH immediately after oleic acid was due to the increase in P_{aCO2} and decrease in HCO_3^- concentration. In the two animals that died after 3.5 and 4 h the sudden decrease in P_{ao} coincided with low values of HCO_3^- concentration (22 and 18 $mmol.l^{-1}$ respectively). We assume that oxygen flux must have been insufficient at that time, leading to metabolic acidosis.

Pulmonary data

The increased weight of the lungs and heart, the infiltrates seen on the chest X-rays and the morphologic evidence indicated pulmonary edema, which was undoubtedly the reason for the decrease in end-expiratory lung volume. Impaired surfactant function, leading to an increase of surface tension in the alveoli causing atelectasis (21), may have contributed to the decrease in V_{EE}. This impaired surfactant function was not coinciding with abnormalities in the lamellar bodies of the pneumocytes II in our experiments and those performed by others (14).

The decrease in total respiratory compliance found in our experiments was due to the decrease in V_{EE}, the formation of pulmonary edema and the impairment of surfactant function after oleic acid. Because we compared C_{rs} before and after oleic acid at corresponding thoracic volume levels, implying a similar effect of thoracic wall recoil forces on intrapulmonary pressure, the decrease in C_{rs} indicated an increased stiffness of the lungs. Hall et al. (21) also found an altered static P-V curve after oleic acid in rabbits.

Hemodynamic variables and heart function

The successive single injections of 0.1 ml oleic acid caused acute rises in P_{pa} (Fig. 7.1). The increase in P_{cv} was probably due to this rise in P_{pa}. We suppose the fall in P_{ao} to be due to a decrease of Q'_t caused by an increase of P_{cv} (19). P_{ao} recovered always within a few minutes, which is in the time domain of the neuro-humoral control mechanisms (32). In our pilot study we have also given larger amounts of oleic acid in one injection. These animals died due to acute right ventricular failure.

The pulmonary hypertension in oleic acid induced lung injury was investigated by others with the determination of pulmonary vascular pressure-flow relationships in dogs, before and after oleic acid (9,35,36). In one of these studies, it was suggested that pulmonary vasoconstriction might be a mechanism for pulmonary hypertension in oleic acid lung injury (36). On the other hand, vascular obstruction by mechanical effects might also be important (1). These mechanisms were confirmed by our histologic and electron microscopical findings

that revealed vasoconstriction, interstitial edema which might cause compression of vessels, intravascular clotting and swelling of endothelial cells.

Q'_t was decreased after oleic acid in our animals, which was also observed by other authors (33,38). It has been suggested (22) that a decrease in venous return, due to a decrease in plasma volume, is the main mechanism for the reduction of cardiac output during oleic acid pulmonary edema. In our experiments P_{cv} was increased which also could have decreased venous return (19). We conclude that transmural central venous pressure ($P_{cv,tm}$) was also increased, because ventilatory conditions were constant, whereas compliance of the chest wall will not have changed (46), resulting in the same intrathoracic pressure at corresponding moments in the ventilatory cycle. An increase in $P_{cv,tm}$ is in contradiction with a decreased venous return to the right ventricle as the primary cause of the fall in cardiac output. Therefore, we consider the increase in P_{pa} to be an important mechanism for the increase in P_{cv} and the reduction in cardiac output. A moderate rise in P_{pa} does not necessarily lead to a decrease in cardiac output (37,43). However, in our experiments the rise in P_{pa} was extensive. Apparently, the heart worked at a higher filling pressure due to such increased afterload on the right ventricle. An additional mechanism that might have contributed to the rise in $P_{cv,tm}$ after oleic acid in our pigs, could be a decreased myocardial contractility, as was demonstrated in dogs (44).

Oxygen delivery

As a result of the decrease in cardiac output and oxygen content in the arterial blood, oxygen delivery was reduced after oleic acid. In two animals severe metabolic acidosis developed after 3.5-4 h indicating an insufficient supply of oxygen to the tissues. Probably, the decrease in D_{O2} after oleic acid is close to a critical value below which maintenance of life is impossible. If this is true, the development of models with a more severe distress will hardly be possible.

Conclusions

A stable model of respiratory distress could be induced in pigs by injecting a series of small doses of oleic acid. The number of injections was different for the different animals. The amount of oleic acid had to be individually adapted, probably depending on the binding capacity of albumine for unsaturated fatty acids. The model fulfilled criteria of respiratory distress and was characterized by 1) a stable P_{aO2} of about 50 mmHg, 2) a severe pulmonary hypertension, 3) a decreased cardiac output resulting in a decreased oxygen delivery, 4) a decreased end-expiratory lung volume and total respiratory compliance, 5) morphologic and

radiographic features of the respiratory distress syndrome, and 6) a stable period of at least 4 hours, allowing studies on basic mechanisms and therapeutic interventions.

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CHAPTER VIII

PULMONARY VASOCONSTRICTION IN OLEIC ACID INDUCED LUNG INJURY. A MORPHOMETRIC STUDY

Pulmonary hypertension is often seen in patients with the adult respiratory distress syndrome (ARDS) (27). In the early phase of this syndrome vasoconstriction is, at least partially, responsible for this increase in pulmonary arterial pressure (P_{pa}). In these patients infusion of nitroprusside decreases P_{pa} whereas cardiac output increases (28). In later stages medial hypertrophy of pulmonary arteries and muscularization of small vessels develops (20,22).

Oleic acid induced lung injury in animals is often used as an experimental model for ARDS (2,8,14,29). The pulmonary hypertension in this animal model was studied by others in dogs, by means of pulmonary vascular pressure-flow relationships (3,14,15). Leeman et al. (15) suggested that active vasoconstriction is a mechanism in pulmonary hypertension after oleic acid administration, as prostaglandin E_1 decreased the driving pressure over the pulmonary circulation considerably.

The aim of the present study was to investigate the distribution and severity of active vasoconstriction in oleic acid induced respiratory distress. Therefore, the muscular pulmonary arteries were analyzed morphometrically in pigs after the induction of respiratory distress with oleic acid. The pulmonary vessels were investigated in both dependent and non-dependent parts of the lungs to asses possible influence of hydrostatic pressure.

METHODS

Surgical procedures

Twelve Yorkshire female pigs (9.3 $\pm$ 0.6 kg) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 $mg.kg^{-1}$). They were placed in supine position on a thermocontrolled operating table to maintain body temperature. Anesthesia was continued by a intravenous infusion of pentobarbital of 8.5 $mg.kg^{-1}.h^{-1}$. The animals were ventilated with a volume controlled ventilator through a tracheal cannula.

Several catheters were inserted: 1) a polythene single lumen catheter into the aortic arch for measuring mean systemic blood pressure (P_{ao}) and sampling of blood, 2) a

Swan-Ganz catheter into the left pulmonary artery for measuring mean pulmonary arterial pressure (P_{pa}), pulmonary blood temperature and sampling of mixed venous blood, 3) a double walled catheter into the right atrium for the injection of saline at room temperature during the estimations of cardiac output (Q'_t) by thermodilution, and 4) a four lumen catheter into the vena cava for measuring mean central venous pressure (P_{cv}) and for infusion of fluids and anesthetics. Each catheter, used for measuring blood pressure, was continuously flushed at a flow rate of 3 ml per hour with normal saline containing 10 I.U. heparine per ml to avoid clotting.

Measured and estimated data

Blood gas tensions and acid-base indices were determined with an automatic blood gas analyzer (ABL3, radiometer). Arterial oxygen saturation (S_{aO2}) and hemoglobine (Hb) concentration were measured with an oxymeter (OSM2, radiometer). Arterial oxygen content was calculated according to:

$$C_{aO2} = [(1.39\ Hb\ S_{aO2})/100] + 0.0031 P_{aO2}$$

where 1.39 is the oxygen binding capacity in ml O_2 per g Hb and 0.0031 is the solubility of oxygen in blood in ml O_2 per 100 ml per mmHg.

P_{ao}, P_{pa} en P_{cv} were continuously measured with use of Baxter disposable pressure transducers, type Uniflow and recorded on a Gould recorder type RS 3800. Pressure values were referred to ambient air pressure and to a zero level corresponding with the level of the manubrium. The transducers were calibrated by application of pressure with a mercury manometer to this reference level.

Q'_t was determined with the thermodilution method (11). Four estimations equally spread over the ventilatory cycle were performed and averaged (12).

Oxygen delivery (D_{O2}) was calculated according to:

$$D_{O2} = C_{aO2} \times Q'_t$$

Ventilation

After the surgical procedures the animals received tubocurarine (0.2 $mg.kg^{-1}$) to suppress spontaneous breathing, and were connected to a computer controlled ventilator, developed at out laboratory (10). Ventilation was set on a frequency of 10 breaths per minute, a tidal volume adjusted to an arterial carbon dioxide tension (P_{aCO2}) of about 40 mmHg, an inspiratory fraction of oxygen of 0.6, an inspiratory to expiratory ratio of 2:3 and a positive end-expiratory pressure of 2 cmH_2O. All settings were kept constant throughout the experiments.

Induction of respiratory distress

After a stabilisation period of about 30 minutes, a baseline period of one hour followed in which measurements of gas exchange and hemodynamic variables were performed. Then respiratory distress was induced in six animals by oleic acid according to a standardized protocol, extensively described elsewhere (Grotjohan et al. A stable model of respiratory distress by small injections of oleic acid in pigs. Submitted for publication). The essentials will be pointed out here. First the animals received 10 ml isotonic dextran-40 per kg body weight intravenously in half an hour via a lumen of the Swan-Ganz catheter and the double walled injection catheter. Next commercial oleic acid (Unichema International, Bebington, United Kingdom, specific gravity 0.89) dissolved 1:1 in 96 % alcohol, was given through the Swan-Ganz catheter in multiple injections of 0.1 ml solution into the right atrium. These injections were given at intervals of 90 s until a stable P_{aO2} below 60 mmHg was achieved. As the injections sometimes had large effects on the circulation, we occasionally lenghtened this interval and halved the dose. Each oleic acid injection was followed by 1 ml saline of 40 °C, in order to flush the catheter.

After the last oleic acid injection the animals were studied for a period varying from 3.5 to 6 h. Two animals died due to metabolic acidosis and circulatory shock at 3.5 and 4 hours respectively. One experiment had to be finished at 5 h after the last oleic acid injection because of technical reasons. The other 3 animals were studied for 6 hours.

Data acquisition and morphology

At the end of the experiments the animals that not died spontaneously (n=4), were killed with a high dose of pentobarbital (0.07 $g.kg^{-1}$). Immediately after death the lungs of all six animals were fixed by instillation of formalin via the trachea. After ligation of blood vessels, heart en lungs were removed en bloc and weighed. From this weight the amount of formalin used for the fixation was subtracted.

Blocks of tissue were taken at four locations of both lungs: ventral and dorsal regions of both apical and diaphragmatic lobe, implying a total number of 8 blocks in each animal. Histologic slides were cut from these blocks and stained with hematoxylin and eosin for morphologic examination and with an elastic-van Gieson stain for the morphometry of the muscular arteries.

The external diameter, without the adventitia, and the thickness of the media were measured in muscular pulmonary arteries. Medial thickness was expressed as a percentage of the external diameter (25). All measurements were carried out by one of the authors (N.W.). Only arteries that were circular or near-circular on cross section and that had a distinct muscular media, between internal and external elastica laminae, were included. From each location 25 muscular arteries, fulfilling these criteria, were measured, giving a total of 200 measurements in each animal.

For electron microscopic investigation, a small block of the ventral part of the left diaphragmatic lobe was taken in some experiments. These small blocks were fixed in

glutaraldehyde solution. After post-fixation with osmium, the blocks were dehydrated with acetone, embedded in LX112, and stained with uranyl acetate and lead citrate.

Six animals were used as controls. They were ventilated for approximately the same time as the animals that received oleic acid.

Statistical analysis

The results were analyzed with student t-tests for paired and unpaired sampels. P-values ≤ 0.05 were considered as statistically significant. Data are presented as mean values $\pm$ 1 sd.

RESULTS

Physiologic data

The effects of the oleic acid injections on gas exchange and hemodynamic variables are presented in Table 8.1, by comparing mean values before and 30 min after the last injection. The total dose that was necessary to obtain a P_{aO2} below 60 mmHg in all animals was 0.12 ± 0.07 ml.kg^{-1}. The multiple oleic acid injections resulted in a stable and low hypoxemia, hypercapnia and decreased pH. In two animals metabolic acidosis developed 3.5 and 4 h after oleic acid administration. These animals died. Dextran infusion decreased Hb concentration from 6.5 ± 0.5 mmol.l^{-1} to 5.0 ± 0.4 mmol.l^{-1} ($p<0.01$). Thereafter Hb concentration increased to 7.6 ± 1.1 mmol.l^{-1} after oleic acid administration. There was a severe stable pulmonary hypertension in all animals accompanied by an elevated central venous pressure, a low stable cardiac output and oxygen delivery and a normal aortic pressure.

Morphology

The weight of the lungs and heart was significantly larger in the oleic acid group as compared to the control group, 33 ± 4 g.kg^{-1} and 21 ± 2 g.kg^{-1} respectively ($p<0.01$).

Histologic examination revealed an extensive acute bronchopneumonia and interstitial edema in all lobes of the animals that received oleic acid. Sometimes interstitial pneumonia was also present. The lesions varied from mild and focal to

Table 8.1. Baseline data and data after oleic acid administration.

	baseline n=6			oleic acid 30 min n=6			
P_{aO2}(mmHg)	292	±	27	57	±	8	*
P_{aCO2}(mmHg)	41	±	3	58	±	7	*
S_{aO2}(%)	99	±	1	74	±	9	*
Hb(mmol.l^{-1})	6.5	±	0.5	7.6	±	1.1	*
Q'_t(ml.s^{-1}.kg^{-1})	2.1	±	0.3	1.4	±	0.2	*
P_{ao}(mmHg)	100	±	11	95	±	12	
P_{pa}(mmHg)	15	±	2	36	±	2	*
P_{cv}(mmHg)	1.5	±	0.8	3.2	±	1.3	*
D_{O2}(ml.s^{-1}.kg^{-1})	32.7	±	4.4	17.4	±	1.4	*
pH	7.46	±	0.04	7.31	±	0.05	*
HCO_3^-(mmol.l^{-1})	28.8	±	1.7	25.6	±	1.8	*

Values are mean ± sd; P_{aO2}, arterial P_{aO2}; P_{aCO2}, arterial P_{CO2}; S_{aO2}, arterial oxygen saturation; Hb, hemoglobine concentration; Q'_t, cardiac output; P_{ao}, arterial pressure; P_{pa}, pulmonary arterial pressure; P_{cv}, central venous pressure; D_{O2}, oxygen delivery; pH, arterial pH; HCO_3^-, standard bicarbonate concentration; * $p \leq 0.01$ compared to baseline.

severe and diffuse. Focal lesions were more prominent than the diffuse lesions. Hyaline membranes were not observed. There was extensive vasculitis with fibrinoid necrosis and fibrin thrombi in some arteries (Fig. 8.1). Pulmonary arteries showed prominent constriction (Fig. 8.2), though varying in severity. There appeared to be no clear differences in the described alterations between the various locations of both lungs.

Electron microscopic investigation revealed intense constriction of muscular arteries with pronounced crenation of the internal elastic lamina and with herniations of the cytoplasm of smooth muscle cells (Fig. 8.3.a and 8.3.b). Sometimes the internal elastic lamina appeared to be swollen. Endothelial cells protruded into the vascular lumen and showed lysosomes (Fig. 8.3.b) and vacuoles as signs of degeneration. The pneumocytes II had a normal aspect.

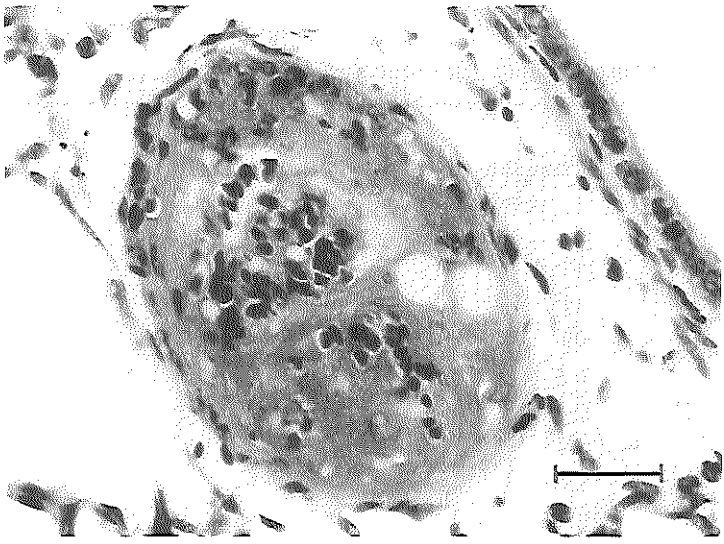

Fig. 8.1 Muscular pulmonary artery with fibrinoid necrosis in pig lung after oleic acid administration. The wall is destroyed and the lumen is filled with a fibrin thrombus. Hematoxylin and eosin, x380. Bar, 40 μm.

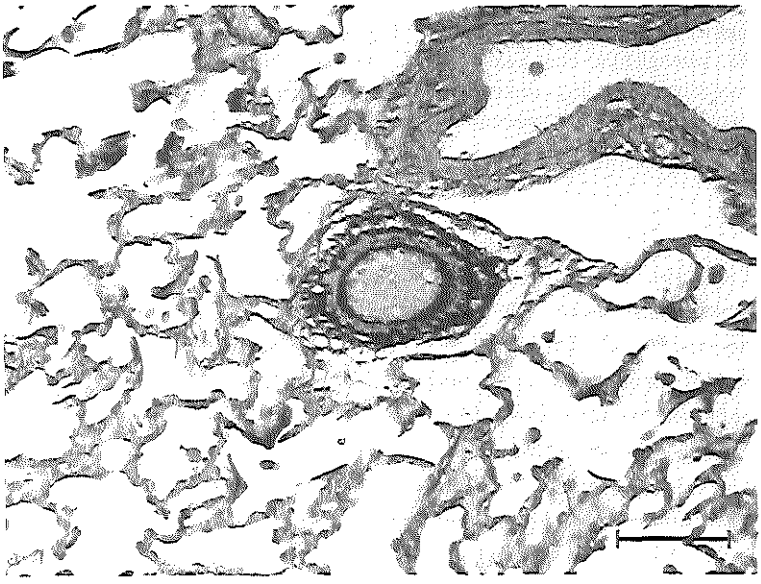

Fig. 8.2. Constricted muscular pulmonary artery in pig lung after oleic acid administration. Pronounced crenation of internal elastic lamina is present. Elastic-van Gieson stain, 150x. Bar, 100 μm.

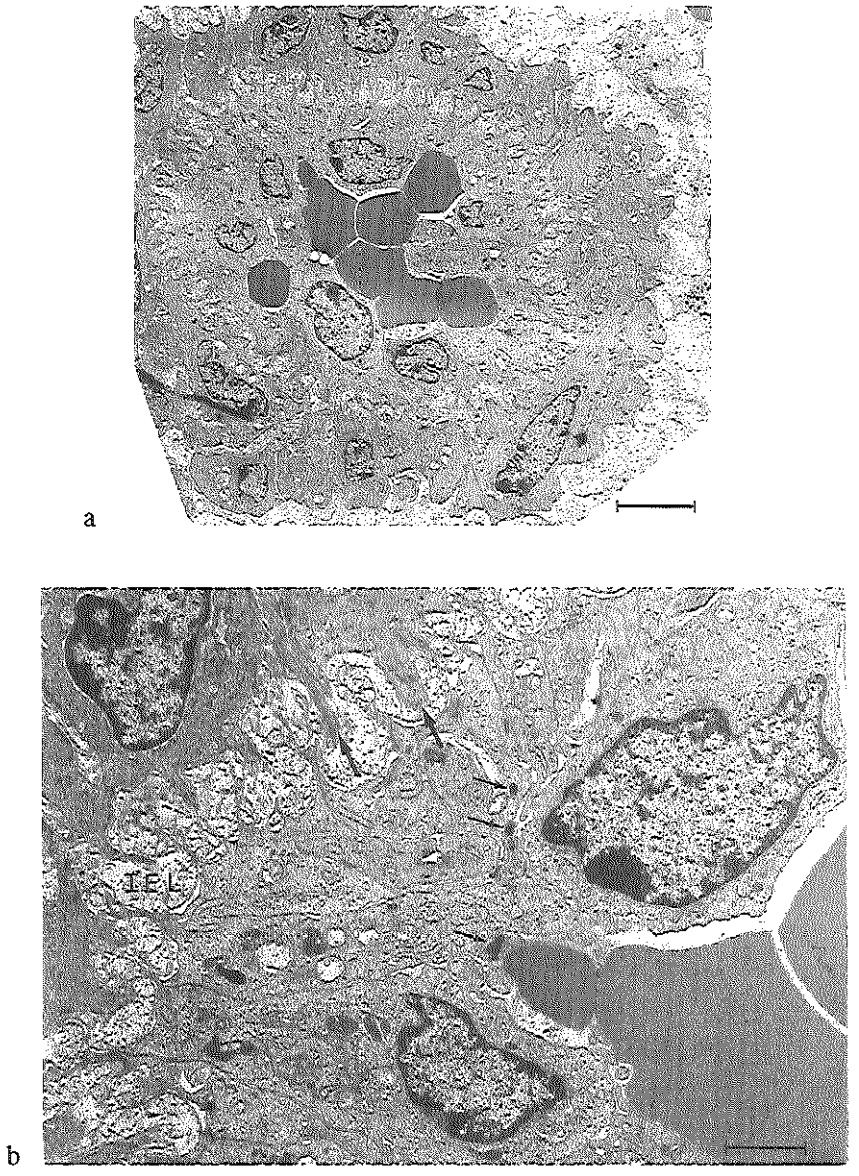

Fig. 8.3. Electron micrograph of lung tissue after oleic acid administration.
a. Severely constricted muscular pulmonary artery with swollen endothelium, protruding into the lumen. Uranyl acetate and lead citrate, 1100 x. Bar, 6 μm. b. Detail: pronounced crenation of internal elastic lamina (IEL) and herniations of cytoplasm of smooth muscle cells (large arrows). Cytoplasm of endothelial cells contains lysosomes (small arrows); 4400 x. Bar, 1.5 μm.

Morphometry

In animals receiving oleic acid, medial thickness of the muscular pulmonary arteries was far greater than in control animals, the overall mean values being 8.1 $\pm$ 3.2 and 3.8 $\pm$ 1.7 respectively ($n=1200$, $p<0.001$). In Fig. 8.4, a frequency histogram of medial thickness of all measured arteries in both control and oleic acid group is presented. There was a distinct overlap between both groups. Mean values for each of the 8 locations in animals of the control and oleic acid groups, are presented in Table 8.2. Besides the mean values, standard deviations of medial thickness in all 8 locations were also greater in the oleic acid group than in the control group (Table 8.2).

Within the oleic acid group there were no significant differences in mean values of medial thickness between apical and diaphragmatic lobe, between ventral and dorsal region and between right and left lung. Within each location, constricted muscular arteries were mostly found in small clusters.

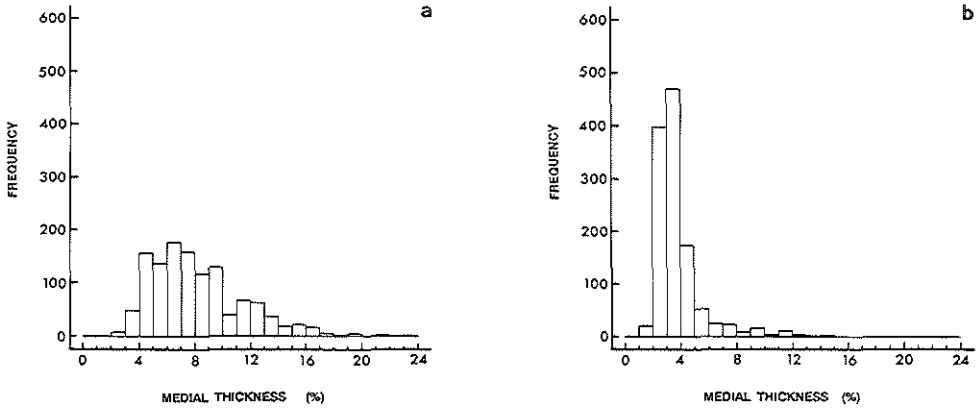

Fig. 8.4. Frequency distribution of medial thickness of muscular pulmonary arteries. a. oleic acid group, b. control group (both $n=1200$). There is a distinct overlap between both groups.

Table 8.2. Medial thickness as percentage of external diameter.

		LA	LD	RA	RD
controls					
1	v:	3.3 (0.9)	2.5 (0.4)	5.2 (2.0)	3.0 (0.7)
	d:	3.2 (0.7)	2.4 (0.3)	9.1 (3.1)	3.4 (0.8)
2	v:	3.4 (0.9)	3.5 (0.7)	5.2 (1.2)	2.7 (0.6)
	d:	3.3 (0.6)	3.5 (0.5)	3.4 (0.7)	3.5 (0.7)
3	v:	3.2 (0.8)	2.9 (0.7)	5.4 (1.3)	2.8 (0.6)
	d:	2.9 (0.8)	3.2 (0.6)	3.2 (0.5)	3.1 (0.7)
4	v:	3.8 (0.8)	3.2 (0.6)	3.9 (1.7)	3.6 (0.7)
	d:	3.3 (0.8)	3.3 (0.9)	4.1 (1.0)	3.1 (0.6)
5	v:	3.5 (0.8)	4.4 (1.4)	3.4 (0.7)	3.3 (0.7)
	d:	3.3 (0.8)	8.5 (2.3)	3.2 (0.7)	3.8 (0.8)
6	v:	3.3 (0.5)	3.7 (0.7)	4.6 (2.0)	3.2 (0.7)
	d:	3.0 (0.5)	7.2 (2.1)	3.0 (0.6)	4.0 (0.8)
oleic acid					
1	v:	9.0 (3.6)	8.3 (4.1)	9.0 (4.8)	9.1 (3.2)
	d:	6.2 (1.7)	7.9 (2.4)	9.1 (3.3)	10.2(4.0)
2	v:	6.3 (2.7)	6.2 (2.3)	7.5 (3.5)	6.9 (2.5)
	d:	4.7 (1.4)	5.5 (1.3)	5.6 (1.3)	6.6 (2.4)
3	v:	11.6(2.3)	10.7(3.9)	9.5 (2.2)	9.9 (2.5)
	d:	11.5(3.1)	11.5(2.4)	8.3 (2.8)	6.7 (1.8)
4	v:	5.1 (1.3)	7.4 (2.0)	8.2 (3.1)	7.6 (2.1)
	d:	8.2 (3.4)	7.7 (2.0)	7.8 (2.3)	7.7 (2.3)
5	v:	8.8 (3.5)	8.0 (1.7)	9.7 (6.0)	6.9 (2.6)
	d:	7.3 (1.2)	7.7 (2.2)	11.7(3.2)	9.1 (2.8)
6	v:	9.5 (3.9)	8.7 (2.0)	8.2 (3.7)	8.2 (2.1)
	d:	5.1 (1.6)	8.4 (2.0)	7.6 (2.7)	8.5 (2.1)

Mean values of 25 measurements per location, sd in brackets. LA and RA: left and right apical lobe, LD and RD: left and right diaphragmatic lobe, v: ventral, d: dorsal.

DISCUSSION

Oleic acid model

By multiple small injections of oleic acid a stable model of respiratory distress for several hours could be induced. We recently described extensively the physiologic effects of oleic acid (Grotjohan et al. A stable model of respiratory distress by small injections of oleic acid in pigs. Submitted for publication). Characteristic features of this model are a low and stable P_{aO2} below 60 mmHg, an increased P_{aCO2}, an increase in Hb concentration, a low and stable Q'_t, a stable pulmonary hypertension, a decreased oxygen delivery to the tissues, a decreased end-expiratory lung volume and a decreased compliance. The increase in lung weight clearly indicates the formation of edema.

Pulmonary hypertension after oleic acid

After the oleic acid administration a severe stable pulmonary hypertension was seen in all animals, the mean P_{pa} being about 36 mmHg. The increase in P_{pa} could be attributed to different mechanisms.

Vasoconstriction. The increase in medial thickness of muscular pulmonary arteries in pigs with oleic acid induced lung injury (Table 8.2) demonstrates the presence of active vasoconstriction. In these short term experiments an increase of arterial smooth muscle mass will not have occurred.

In dogs with oleic acid induced lung injury, the pulmonary circulation was analyzed by means of pressure-flow curves before and after prostaglandin E_1 administration (15). It was stated that the pulmonary hypertension could be partly due to vasoconstriction, as prostaglandin E_1, a vasodilator, reduced the pulmonary hypertension after oleic acid application considerably.

Intravascular obstruction. The swelling of endothelial cells, protruding into the vascular lumen (Fig. 8.3.b), and the presence of microthrombi and fibrinoid necrosis (Fig. 8.1) in the pulmonary vessels of our pigs, indicate that intravascular obstruction could have contributed to the increase in P_{pa}. Velazquez and Schuster (24) claimed a decrease in lobar perfusion in dogs already 1 min after the infusion of oleic acid, while hypoxic pulmonary vasoconstriction was blocked with endotoxine. They concluded that intravascular obstruction was, at least in the early phase, the main cause for the observed redistribution of blood

flow.

Vascular compression. Another mechanism that might have contributed to the increase in P_{pa} in our pigs, could be compression of pulmonary vessels due to edema. The increased weight of lungs and heart in these animals, clearly indicated the development of edema. Interstitial edema was also demonstrated histologically. It has been shown that in dogs interstitial edema per se does not compress pulmonary arteries, as the diameter and area were not decreased in this situation (16). In another study however, unilobar edema in dogs after endobronchial instillation of hypotonic plasma, leading to alveolar flooding, reduced perfusion to the edemateous lobe (1).

Pulmonary vasoconstriction

Vasoconstriction of the muscular pulmonary arteries may be caused by hypoxia and vasoactive substances released by granulocytes (4,21) and other cells. Among the various species, the pig has one of the greatest pulmonary vasoconstrictor responses to hypoxia (23). We found extensive numbers of neutrophils in the lungs of the pigs that received oleic acid. This finding was also documented by others (5,7,9,18).

It has been demonstrated in dogs that alcohol, given in a dose 4 to 6 times greater than the dosage used in our experiments, could potentiate hypoxic pulmonary vasoconstriction (6). In our experiments, the amount of alcohol was too small for such an effect. Moreover, it will have been metabolized completely within two hours after the first injection (17).

Pentobarbital can attenuate vascular tone of pulmonary vessels in high doses (26). The morphometric data of the two animals that died spontaneously, were not different from those in the four animals that received a high lethal dose of pentobarbital. Therefore, we assume that this lethal dose did not affect pulmonary vasoconstriction in our animals to a great extent.

Statistical differences in medial thickening between different locations did not occur. Dependent and non-dependent regions had a similar pattern of vasoconstriction. Some authors reported a redistribution of blood flow from dorsal to mid and ventral regions after oleic acid administration in dogs in supine position (19). There appeared to be no direct relation with the formation of edema. Our morphometric data do not provide evidence for such a redistribution of blood flow from dependent to nondependent regions in our pigs. Moreover, we did not find any differences in histologic features between the dependent and non-dependent regions.

A reason for the redistribution of blood flow from dependent to nondependent lung areas in dogs after oleic acid found by other authors (19), whereas we found no evidence for this phenomenon, could be the method of infusion of oleic acid. In the dog study undiluted oleic acid was injected in three bolus injections. We dissolved the oleic acid in 96 % alcohol to obtain a homogenous solution and administered the oleic acid in multiple small injections.

Not only were mean values of medial thickness of all eight locations increased in the oleic acid group, variation in medial thickness within each location was also higher than in the control group (Table 8.2), implying constricted vessels next to vessels with normal vascular tone. The observed clusters of actively constricted arteries were probably due to the constriction of large muscular arteries together with their branches. The variation in vasoconstriction within each location might be related to the predominantly focal inflammatory lesions that we found in our pigs. This patchy pattern of morphologic alterations was also described by others (5,9,13). The focal lesions probably resulted in local differences in hypoxia, causing local vasoconstriction to match perfusion to ventilation.

Conclusions

By analyzing the morphometric data of muscular pulmonary arteries in pigs with oleic acid induced lung injury, we found severe active vasoconstriction, which was inhomogeneously distributed in all locations investigated. There were no differences in pattern of vasoconstriction between the right and left lungs and between dependent and non-dependent lung regions. Morphologic investigation revealed that, besides active vasoconstriction, endothelial swelling and intravascular clotting might contribute to the early stage of pulmonary hypertension in oleic acid induced pulmonary edema.

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CHAPTER IX

APPLICATION OF PEEP IN OLEIC ACID INDUCED LUNG INJURY. WHICH INDICATOR PREDICTS MAXIMAL OXYGEN DELIVERY?

To maintain sufficient gas exchange in patients with adult respiratory distress syndrome (ARDS), mechanical ventilation with positive end-expiratory pressure (PEEP) is often necessary. Since 1967 (1) PEEP has been applied in the treatment of ARDS patients. PEEP effects were analyzed in many studies in patients (4,5,11,15,18,29,32,48) as well as in animals (13,40) to define indicators for determining an appropriate level of PEEP. Some authors referred to compliance as an indicator (48), which was not supported by others (15, 40). Arterial to end-tidal carbon dioxide gradient was proposed in an animal study (40), which was confirmed (4) and rejected (29) for application in patients. Other suggestions were to use P_{aO2} (5), venous admixture (11,18,32) or end expiratory lung volume as an indicator (13). A main reason for the conflicting results between the different patient and animal studies could be differences in conditions.

Therefore, we aimed at the evaluation of variables, which could serve as indicators of the optimal level of PEEP, under the same conditions, consisting of early respiratory distress in pigs, induced by oleic acid. We regarded the level of PEEP to be optimal when oxygen delivery was maximal, as Suter et al. (48) did.

METHODS

Surgical procedures

Seven female young pigs (5-7 weeks old, 9.5 $\pm$ 0.9 kg) were anesthetized with pentobarbital sodium (30 $mg.kg^{-1}$ intraperitoneal) and placed in supine position. Body temperature was maintained at about 38°C. Tracheostomy was performed and the pigs were connected to a volume controlled ventilator. Four catheters were inserted: 1) a polythene single lumen catheter into the aortic arch, for measuring arterial blood pressure (P_{ao}) and sampling of blood, 2) a Swan-Ganz catheter into the left pulmonary artery for monitoring pulmonary arterial pressure (P_{pa}) and blood temperature and for sampling of mixed venous blood, 3) a double walled catheter into the right atrium for the injection of saline at room temperature during the thermodilution procedures and 4) a four lumen catheter into the superior vena cava for measuring central venous pressure (P_{cv}) and for

the infusion of fluids and anesthetics. With the exception of the double walled catheter, all catheters were continuously flushed with saline, containing 10 I.U. heparine per ml, at a flow rate of 3 ml per hour to avoid clotting in the catheters. To avoid retention of urine, a catheter was put into the urinary bladder.

At the end of the surgical procedures, the animals received tubocurarine (0.2 mg. $kg^{-1}.h^{-1}$) to suppress spontaneous breathing. Next mechanical ventilation was continued with a computer controlled ventilator, developed at our laboratory (26). This ventilator consisted of two separate in parallel functioning bellows, one for continuous ventilation during the experiments, and the other for determination of end-expiratory lungvolume.

Measurements, estimations and data aquisition

Gas exchange and hemoglobine concentration

For measuring oxygen and carbon dioxide tensions and acid-base indices of blood, an automatic blood gas analyzer (Radiometer ABL3) was used. Hemoglobin (Hb) concentration and O_2 saturation were determined with use of an oxymeter (Radiometer OSM2). A Perkin-Elmer mass spectrometer (MGA 1100) was used to analyze inspiratory and mixed expiratory gases, including helium (He).

The in- and expiratory carbon dioxide tension was continuously monitored with an infrared analyzer (Hewlett-Packard capnometer, type 47210A). From this capnogram the end tidal P_{CO2} (P_{etCO2}) was obtained. Airflow (V') was recorded with use of a Godart pneumotachograph, type nr. 0.

Arterial oxygen content (C_{aO2}) was calculated according to:

$$C_{aO2} = (1.39\ Hb\ S_{aO2})/100 + 0.0031\ P_{aO2}$$

where 1.39 is oxygen binding capacity in ml O_2 per g Hb, S_{aO2} is arterial oxygen saturation in %, 0.0031 is solubility of oxygen in blood in ml O_2 per 100 ml per mmHg, and P_{aO2} is arterial oxygen tension in mmHg. Mixed venous oxygen content ($C_{\bar{v}O2}$) was calculated in the same way, using mixed venous oxygen saturation ($S_{\bar{v}O2}$) and mixed venous oxygen tension ($P_{\bar{v}O2}$).

Venous admixture (Q'_s/Q'_t) in percentage of total pulmonary blood flow (Q'_t) was calculated according to (3):

$$Q'_s/Q'_t = 100\ (C_{cO2}-C_{aO2})/(C_{cO2}-C_{\bar{v}O2})$$

where C_{cO2} is pulmonary end-capillary oxygen content in ml O_2 per 100 ml blood. The alveolar oxygen tension (P_{AO2}) was used as a substitute for the pulmonary end-capillary oxygen tension. P_{AO2} was derived from the alveolar gas equation:

$$P_{AO2} = P_{IO2} - P_{ACO2}\ [F_{IO2} + (1-F_{IO2})/R]$$

where P_{IO2} and F_{IO2} are the inspiratory oxygen tension and fraction. P_{ACO2} is the alveolar carbon dioxide tension, which we assumed to be equal to P_{aCO2}. R is the respiratory quotient.

The pulmonary end-capillary oxygen saturation was determined with use of the P_{AO2} and the oxygen saturation curve of pig blood. The parameters in the equation of the human oxygen saturation curve (31) were fitted for the pig's oxygen saturation curve, based on pig blood data from other experiments in our laboratory. This oxygen saturation curve was in accordance with the curve described by Bartels and Harms (2).

Physiological dead space (V_D/V_T) in percentage of tidal volume was obtained from the equation (14):

$$V_D/V_T = 100\ (P_{aCO2} - P_{\bar{E}CO2})/P_{aCO2}$$

where $P_{\bar{E}CO2}$ is the mixed expiratory carbon dioxide tension.

Oxygen uptake (V'_{O2}) was calculated from:

$$V'_{O2} = (V'_I \times F_{IO2}) - (V'_E \times F_{\bar{E}O2})$$

where V'_I is the inspiratory airflow rate, V'_E the expiratory airflow rate and $F_{\bar{E}O2}$ is the mixed expiratory oxygen fraction.

Pulmonary data

End-expiratory lung volume

End-expiratory lung volume (V_{EE}) was estimated with use of an open He wash-in and wash-out technique (24). During 90 seconds the lungs were ventilated with a gas mixture containing 4-5 % He to wash-in the He, after changing ventilation at end-expiration to the second bellows of the ventilator. After this period, ventilation was resumed with the initial gas mixture to perform the He wash-out. He fractions were continuously measured by the mass spectrometer during the whole procedure. During the He wash-in, V_{EE} could be determined at the end of each expiration from 1) the inspired amount of He since the start of the wash-in, 2) the expired amount of He since the start of the wash-in, and 3) the He fraction in the lung after the last expiration, which we assumed to be equal to the measured fraction at the end of that last expiration, $F_{EE,He}$.

V_{EE} was calculated according to the mass balance:

$$V_{EE} = (\int V'.F_{I,He}.dt - \int V'.F_{E,He}.dt)/F_{EE,He}$$

where V' is the airflow rate, $F_{I,He}$ is the inspiratory fraction of He, $F_{E,He}$ is the expiratory fraction of He. For the calculation of V_{EE} during the He wash out, a similar formula was used. For the presentation of the data the values of V_{EE} at the end of the wash-in and wash-out were used.

Total respiratory compliance

Compliance of lungs and thorax (C_{rs}) was estimated with use of ventilatory maneuvers with an inspiratory pause. Tracheal pressure (P_T) was measured in the tracheal cannula with a fluid filled catheter, provided with side holes, and connected to a Baxter disposable pressure transducer type Uniflow. Inspiratory volumes of 6, 12 and 18 ml per kg body weight were injected at intervals of 2 min. Each insufflation was followed by a pause of 3 s. During these pauses tracheal pressure and thoracic volume decreased gradually. The volume change during a pause was recorded with use of a mercury cord, which was fixed around the thorax at 5 cm cranial from the sternal xyphoid. Volume and pressure at the end of the inspiratory pause served as the data for the compliance estimation. A third degree polynomial pressure-volume curve was fitted through these data and through end-expiratory pressure and volume. This P-V curve implied an approximately linear part between the volumes 4 and 8 $ml.kg^{-1}$. The slope of this part was used as the compliance estimate (25). At each level of PEEP, we also calculated total respiratory compliance by dividing the insufflated volume of one pause, 12 $ml.kg^{-1}$, by its pause pressure. This compliance value is specified as C_p.

The mercury cord was calibrated with the three insufflated volumes mentioned above. Besides changes in thoracic volume during a pause procedure, changes in thoracic volume during the application of PEEP could also be monitored with the mercury cord. These changes in thoracic volume were correlated with the changes in V_{EE}, estimated with the He wash-in and wash-out technique. The stability of the mercury cord was tested during a period of ten hours by alternate stretch and release to an amount corresponding with ventilation and at a rate of 10 per minute. We did not observe any change in zero level and gain.

Hemodynamic data and oxygen delivery

P_{ao}, P_{pa} and P_{cv} were measured continuously using disposable pressure transducers (Baxter, type Uniflow). Pressure values were referred to ambient air pressure and to a zero level at the height of the manubrium. Transducers were calibrated by application of pressure to this reference level under guidance of a mercury manometer. All blood pressures are presented as mean values over a ventilatory cycle.

Cardiac output (Q'_t) was determined with the thermodilution method (27). Four determinations, equally spread over the ventilatory cycle, were performed and averaged. This averaged value can be considered as an accurate estimate of mean cardiac output (28).

Oxygen delivery (D_{O2}) and systemic vascular resistance (R_{sys}) were calculated according to:

$$D_{O2} = C_{aO2} \times Q'_t$$

$$R_{sys} = (P_{ao}-P_{cv})/Q'_t$$

Signal processing

Throughout the experiments ECG, all blood pressures, P_T, V', the resistance of the mercury cord and pulmonary blood temperature were continuously recorded on a Gould recorder type RS 3800. During the estimations of Q'_t all other hemodynamic signals were sampled (250 Hz) on line by a computer for a period of 3 ventilatory cycles. P_T, V' and the mercury cord signal were sampled for 9 s at 100 Hz during each pause procedure of the C_{rs} estimation. During the determination of V_{EE}, the in- and expiratory gases were sampled by a computer at 50 Hz. All signals were also stored on a Racal thermionic store 14, electromagnetic tape recorder.

Substitute of changes in intrathoracic pressure

During the application of PEEP, changes in P_{cv} correlate closely with changes in intrathoracic pressure in pigs with normal lungs (44). We studied this correlation in an additional group of 5 pigs, which received oleic acid in a comparable protocol as we performed in the present PEEP study. Intrathoracic pressure (P_{it}) was measured with a fluid-filled catheter (0.6 mm ID) which was placed air tight via a needle through the right fourth intercostal space in the intrapleural space. The presence of an air-fluid interface was avoided. Both P_{cv} and P_{it} were measured with use of Statham P23De transducers. The highest level of PEEP used was a PEEP of 12 cmH_2O.

Ventilatory conditions and induction of respiratory distress

During a stabilisation period of about 30 minutes after the operation, tidal volume was adjusted to a P_{aCO2} of about 40 mmHg. Other ventilatory settings were: a PEEP of 2 cmH_2O, an F_{IO2} of 0.6, an I:E ratio of 2:3, and a ventilatory cycle of 6 s. These ventilatory conditions were maintained during the stepwise increases of PEEP.

In a period of one hour baseline measurements of gas exchange and hemodynamic variables were performed. Then respiratory distress was induced in all animals by oleic acid administration, according to a standardized protocol (20). First the animals received 10 ml isotonic dextran-40 per kg body weight. Next commercial oleic acid (Unichema International, Bebington, United Kingdom, specific gravity 0.89) dissolved 1:1 in 96 % alcohol, was given through the Swan-Ganz catheter in multiple injections of 0.1 ml solution into the right atrium. These injections were given at intervals of 90 s until a stable P_{aO2} below 60 mmHg was achieved. As the injections sometimes had large effects on the circulation, we occasionally lenghtened this interval and halved the dose. Each oleic acid injection was followed by 1 ml saline of 40 °C, in order to flush the catheter.

PEEP-protocol

To observe whether a stable respiratory distress was established, all animals were monitored during 1 h after the last oleic acid injection. One animal was excluded from the study because of deterioration of gas exchange and development of metabolic acidosis in the first hour. In 6 animals PEEP was raised in steps of 2 cmH_2O to a maximum of 12 cm H_2O, each step lasting 30 min. In our notation the level of PEEP is specified by a subscript, e.g. $PEEP_4$ is a PEEP of 4 cmH_2O. In the last 15 min of each PEEP step measurements of gas exchange, V_{EE}, C_{rs} and hemodynamics were performed. One animal died shortly after $PEEP_{10}$, due to circulatory failure. After all steps, PEEP was returned to $PEEP_2$ in 10 min. Twenty minutes later all measurements were done again. In one animal not all measurements could be performed when $PEEP_2$ was regained, because it died suddenly after the arterial blood sampling.

Control data were obtained from a previous study (20), in which the animals were observed for 6 h after oleic acid administration without any intervention.

Statistical analysis

The results were analyzed using standard repeated measures analysis of variance or student t-tests for paired and unpaired samples. We calculated Pearson's correlation coefficients. Our statistical analyses were done with use of SPSS and Statgraphics. P-values ≤ 0.05 were considered as statistically significant. Data are presented as mean values $\pm$ 1 sd.

RESULTS

Changes in intrathoracic pressure

A good correlation was found between changes in intrathoracic and changes in central venous pressure in the additional study with 5 pigs after oleic acid administration (Fig. 9.1, $p<<0.001$, $r=0.93$). These data encouraged us to calculate changes in transmural pulmonary arterial pressure ($P_{pa,tm}$) during PEEP by subtracting changes in P_{cv} from changes in P_{pa}.

Conditions before and after oleic acid administration

The total amount of administered oleic acid was 0.13 ± 0.04 $ml.kg^{-1}$, which was similar to that in our previous study (0.12 ± 0.07 $ml.kg^{-1}$).

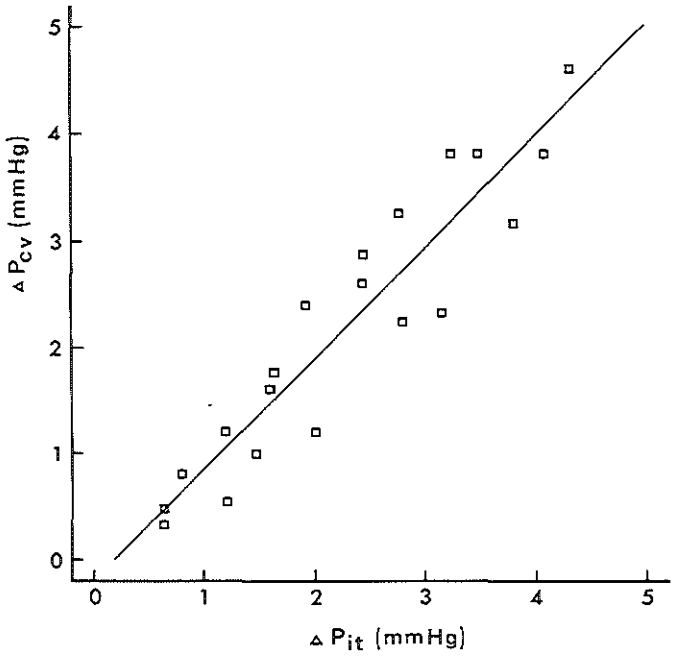


Fig. 9.1. Changes in central venous and intrathoracic pressure.
Changes in central venous pressure (ΔP_{cv}) and changes in intrathoracic pressure (ΔP_{it}) correlated significantly ($r=0.93$, $p<<0.001$) during the application of PEEP in 5 pigs after oleic acid administration. Regression equation: $\Delta P_{cv} = -0.2 + 1.05\ \Delta P_{it}$.

Variables of gas exchange, end-expiratory lung volume and total respiratory compliance before oleic acid administration, 15 min after oleic acid administration, 60 min after oleic acid administration just before the start of the PEEP protocol and after the PEEP procedures are presented in Table 9.1. As mentioned, the data of a previous study served as control values. The multiple oleic acid injections resulted in a stable hypoxemia, increased venous admixture and hypercapnia, as evident from the two columns at 15 and 60 min after the last oleic acid injection. Hb concentration increased in spite of the preceding administration of dextran. Both V_{EE} and C_{rs} were decreased. The hemodynamic variables and oxygen delivery are presented in Table 9.1.

Table 9.1. Data before and after oleic acid administration.

		baseline n=6	oleic acid (15 min) n=6	oleic acid (60 min) before PEEP procedures n=6	oleic acid after PEEP procedures n=5
P_{aO2} (mmHg)	P	310 ± 26	54 ± 7	56 ± 7	63 ± 7 *
	C	292 ± 27	58 ± 13	55 ± 6	52 ± 4
S_{aO2} (%)	P	99 ± 1	72 ± 6	76 ± 7	77 ± 6
	C	99 ± 1	74 ± 10	72 ± 7	65 ± 13
$P_{\bar{v}O2}$ (mmHg)	P	45 ± 3	29 ± 2	32 ± 1	34 ± 4 (n=4)
	C	49 ± 2	29 ± 4	30 ± 3	32 ± 5 (n=4)
$S_{\bar{v}O2}$ (%)	P	67 ± 7	27 ± 3	30 ± 4	31 ± 6 (n=4)
	C	69 ± 5	24 ± 4	25 ± 5	29 ± 6 (n=4)
P_{aCO2} (mmHg)	P	42 ± 3	65 ± 9	65 ± 8	71 ± 6
	C	41 ± 3	55 ± 6	58 ± 9	66 ± 14 #
Q'_s/Q'_t (%)	P	6 ± 2	42 ± 8	39 ± 9	37 ± 7 (n=4)
	C	7 ± 2	43 ± 6 (n=5)	43 ± 6 (n=5)	43 ± 9 (n=3)
V_D/V_T (%)	P	35 ± 6	62 ± 4	60 ± 5	64 ± 6 (n=4)*
	C	34 ± 11	58 ± 5 (n=5)	58 ± 3 (n=5)	53 ± 3 (n=3)
Hb ($mmol.l^{-1}$)	P	6.3 ± 0.6	7.1 ± 0.8	7.2 ± 1.0	7.1 ± 0.8
	C	6.5 ± 0.5	7.4 ± 1.0	7.7 ± 1.2	8.1 ± 1.2
pH	P	7.47 ± 0.04	7.29 ± 0.05	7.29 ± 0.06	7.23 ± 0.06 #
	C	7.46 ± 0.04	7.33 ± 0.04	7.32 ± 0.06	7.27 ± 0.09 #
HCO_3^- ($mmol.l^{-1}$)	P	30.7 ± 1.4	26.5 ± 1.3	27.1 ± 1.6	23.9 ± 2.9
	C	28.8 ± 1.7	25.6 ± 1.5	25.6 ± 2.2	24.5 ± 3.7
P_{aCO2}-P_{etCO2} (mmHg)	P	4 ± 2	29 ± 5	27 ± 6	37 ± 9
	C	3 ± 2	22 ± 5 (n=5)	21 ± 6	27 ± 7

V_{EE}	P	25	±	3	*		-			16	±	3	*	21	±	3 ##
(ml.kg^{-1})	C	21	±	3			-			11	±	3		12	±	3 (n=3)
C_{rs}	P	1.7	±	0.1			-			0.9	±	0.2		1.0	±	0.2(n=4)
(ml.cmH_2O^{-1}.kg^{-1})	C	1.8	±	0.3			-			0.8	±	0.3		0.8	±	0.4(n=3)
Q'_t	P	2.1	±	0.3		1.6	±	0.2		1.6	±	0.2		1.6	±	0.3(n=4)
(ml.s^{-1}.kg^{-1})	C	2.1	±	0.3		1.4	±	0.3 (n=5)		1.4	±	0.1		1.4	±	0.2
P_{ao}	P	89	±	8		92	±	14		91	±	13		60	±	18
(mmHg)	C	100	±	11		95	±	13		98	±	14		91	±	36
P_{pa}	P	12	±	1		36	±	3		36	±	4		31	±	3 #
(mmHg)	C	15	±	2		35	±	2		38	±	2		34	±	3
P_{cv}	P	0.9	±	0.5		2.8	±	1.2		2.5	±	1.6		2.2	±	1.4
(mmHg)	C	1.5	±	0.8		3.4	±	1.0		3.4	±	1.4		2.9	±	2.0
D_{O2}	P	32	±	6		18	±	3		20	±	3		20	±	3 (n=4)
(ml.s^{-1}.kg^{-1})	C	33	±	4		16	±	2 (n=5)		18	±	1		18	±	5
HR	P	161	±	45		180	±	42		201	±	46		224	±	23 (n=4)
(beats.min^{-1})	C	181	±	60		156	±	38		179	±	42		221	±	34 #
R_{sys}	P	42	±	4		57	±	9		57	±	9		41	±	6 (n=4)##
(mmHg.ml^{-1}.s.kg)	C	47	±	3		66	±	21 (n=5)		66	±	16		60	±	26

P, present study; C, added data of control group; mean values ± sd; where n is different from n in the headline, measurements failed because of technical reasons. P_{aO2}, arterial oxygen tension; S_{aO2}, arterial oxygen saturation; $P_{\bar{v}O2}$, mixed venous oxygen tension; $S_{\bar{v}O2}$, mixed venous oxygen saturation; P_{aCO2}, arterial carbon dioxide tension; Q'_s/Q'_t, venous admixture; V_D/V_T, physiological dead space; Hb, hemoglobine concentration; pH, arterial pH; HCO_3^-, arterial bicarbonate concentration; P_{aCO2}-P_{etCO2}, arterial minus end-tidal P_{CO2}; V_{EE}, end-expiratory lung volume (He wash-in); C_{rs}, total respiratory compliance; Q'_t, cardiac output; P_{ao}, arterial blood pressure; P_{pa}, pulmonary arterial pressure; P_{cv}, central venous pressure; D_{O2}, oxygen delivery; HR, heart rate; R_{sys}, systemic vascular resistance. All variables at 15 and 60 min after oleic acid administration were significantly different from their baseline values, except P_{ao} and HR ($p \leq 0.01$; R_{sys}, $p < 0.05$); * $p < 0.05$, compared to control; # $p < 0.05$ and ## $p < 0.01$, compared with the value immediately before the PEEP procedures.

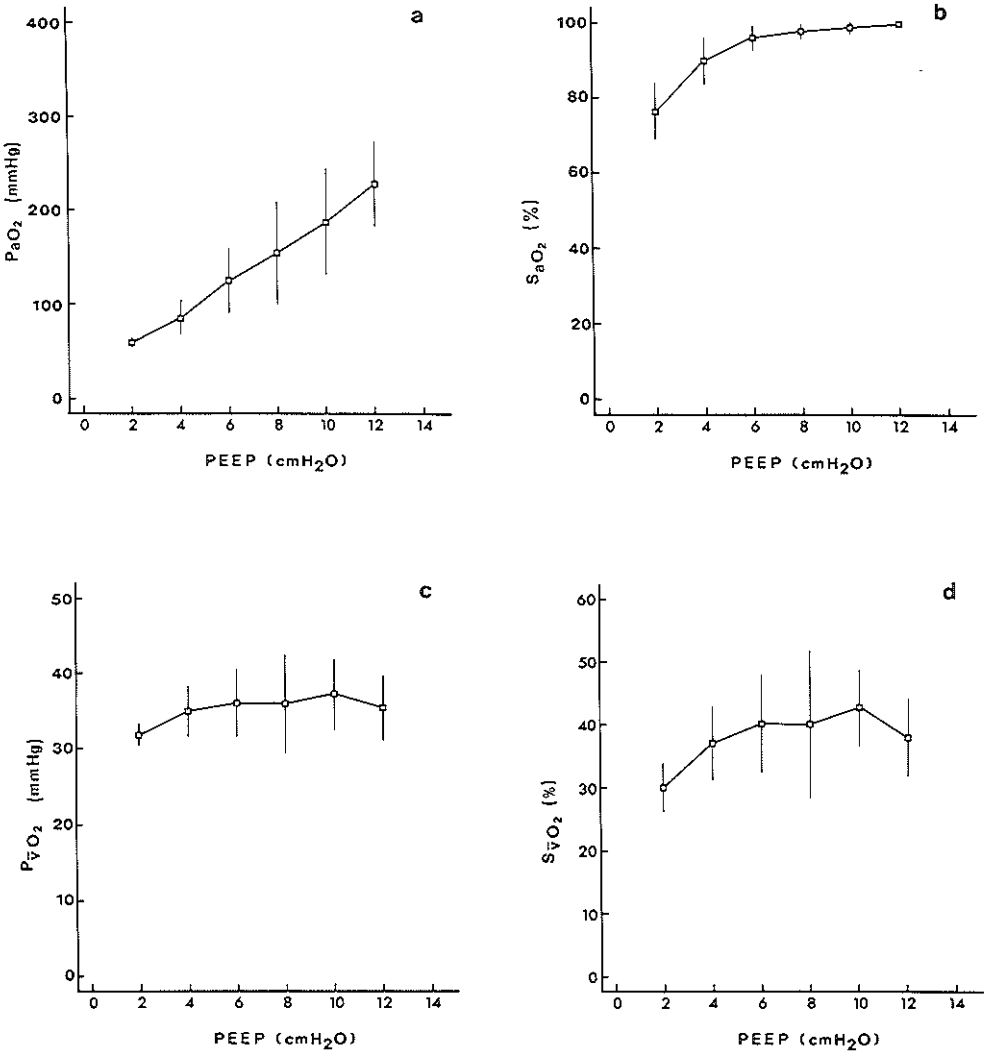
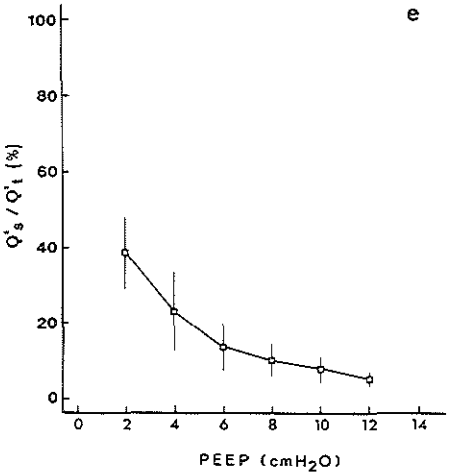


Fig. 9.2. Oxygen exchange variables related to PEEP.
Mean values plotted against positive end-expiratory pressure (PEEP), n=6; $PEEP_{10}$ and $PEEP_{12}$ n=5. Vertical bars: sd. a. arterial oxygen tension, b. arterial oxygen saturation, c. mixed venous oxygen tension, d. mixed venous oxygen saturation, e. venous admixture.



A severe pulmonary hypertension with an elevated central venous pressure, a low and stable cardiac output, a normal aortic pressure and an increased systemic vascular resistance were found after the oleic acid injections. Oxygen delivery was markedly reduced. Except for V_{EE}, no statistical differences in the first hour after the oleic acid injections were present between the PEEP and the control group. The reduction in V_{EE} by oleic acid administration, however, was the same in both groups.

PEEP application

Gas exchange and Hb concentration

The application of PEEP increased P_{aO2} linearly in all animals from 54 $\pm$ 7 mmHg to 226 $\pm$ 45 mmHg at $PEEP_{12}$ ($p<0.01$, Fig. 9.2.a). S_{aO2} increased from 76 $\pm$ 7 % to 99 $\pm$ 1 ($p<0.01$, quadratic trend, Fig. 9.2.b). $P_{\bar{v}O2}$ increased with the rise of PEEP up to $PEEP_{10}$ and was decreased again at $PEEP_{12}$ (Fig. 9.2.c). The test for a quadratic trend was just not significant ($p=0.053$). $S_{\bar{v}O2}$ changed according to the same pattern as $P_{\bar{v}O2}$ ($p<0.05$, quadratic trend, Fig. 9.2.d). Moreover Q'_s/Q'_t decreased with each step of PEEP ($p<0.01$, quadratic trend, Fig. 9.2.e).

P_{aCO2} decreased slightly but significantly to 59 ± 11 mmHg at $PEEP_{12}$ ($p<0.05$ linear trend, Fig. 9.3.a). The P_{aCO2}-P_{etCO2} gradient (linear trend) and V_D/V_T (linear and quadratic trend) decreased too with each step of PEEP ($p<0.01$, Figs. 9.3.b and 9.3.c respectively).

Hb concentration, pH, standard bicarbonate concentration (HCO_3^-) and V'_{O2} did not change during the application of PEEP.

When PEEP was returned to 2 cmH_2O, all gas exchange variables, except for pH, were not different from their values before the application of PEEP (Table 9.1).

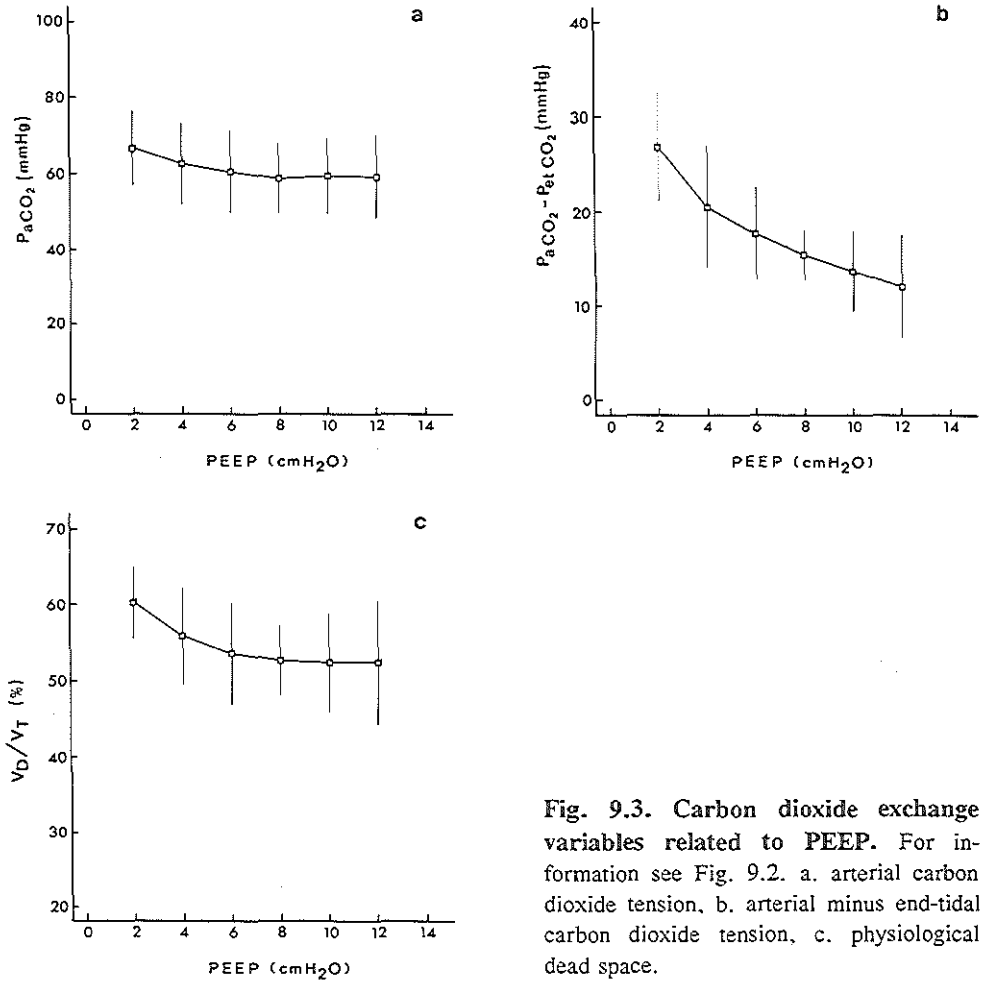


Fig. 9.3. Carbon dioxide exchange variables related to PEEP. For information see Fig. 9.2. a. arterial carbon dioxide tension, b. arterial minus end-tidal carbon dioxide tension, c. physiological dead space.

P_{aO2} and V_D/V_T were slightly but significantly higher than the corresponding control values ($p<0.05$).

Pulmonary data

End-expiratory lungvolume

V_{EE} increased with each level of PEEP ($p<0.01$, linear, quadratic and cubic trend, Fig. 9.4). The values of the He wash-in and wash-out correlated closely ($p<0.001$, $r=0.99$). Moreover a high correlation between changes in V_{EE} and changes in thorax volume measured with the mercury cord was found during the PEEP application ($r=0.98$, $p<<0.001$). After PEEP application V_{EE} remained at a significantly higher level compared with its value before PEEP ($p<0.01$, Table 9.1). It was also significantly different from the control value corresponding in time ($p<0.01$). In the control group where $PEEP_2$ was maintained, no changes in V_{EE} occurred over a period of 3 hours. In one animal no He wash-in and wash-out could be performed at $PEEP_2$ after the PEEP procedures, because this animal died shortly after the arterial blood sampling. In this animal V_{EE} was estimated with use of the mercury cord.

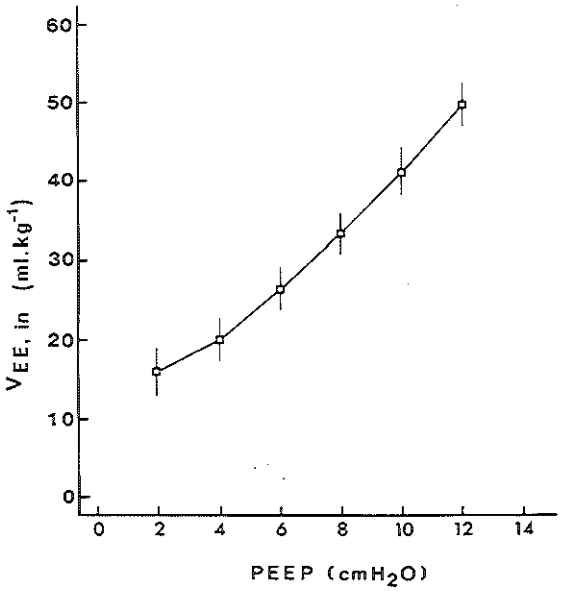


Fig. 9.4. End-expiratory lung volume vs PEEP. For information see Fig. 9.2. End-expiratory lung volume was determined with an open He wash-in method.

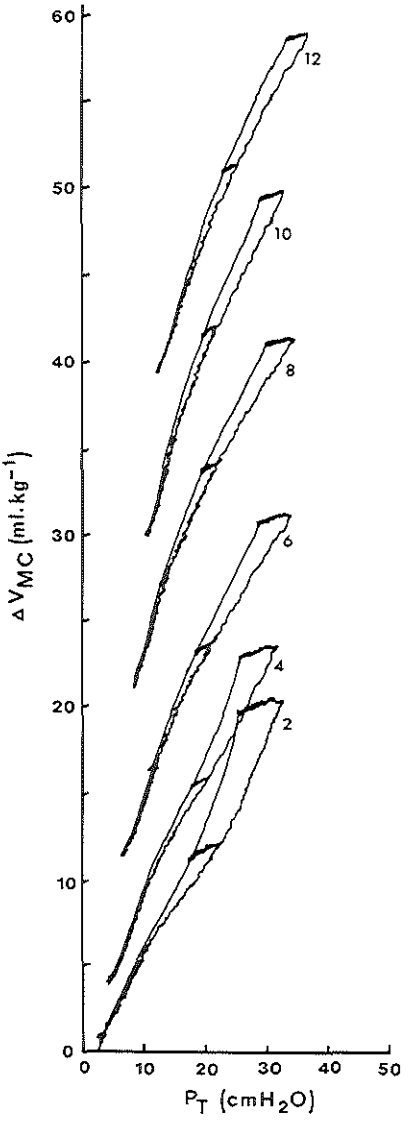


Fig. 9.5. Pressure-volume curves. Pressure-volume (P-V) curves of an individual experiment at increasing levels of PEEP, indicated by a number at the top of each curve (2=$PEEP_2$ etc.). ΔV_{mc}, change in thoracic volume, measured with a mercury cord. The zero level corresponds with the end-expiratory volume at $PEEP_2$ after oleic acid administration. P_T, tracheal pressure.

Total respiratory compliance

An individual example of the effect of PEEP on the P-V curve is presented in Fig. 9.5. Each stepwise increase in PEEP shifted the constructed P-V curve upwards. C_{rs} was increased up to the highest level of PEEP.

The effect of PEEP on C_{rs} of all six animals is presented in Fig. 9.6. From $PEEP_2$ to $PEEP_4$, C_{rs} decreased in three animals, whereas in the other three animals C_{rs} increased. Mean values were not statistically different. From $PEEP_4$ an increase in the averaged C_{rs} was found when PEEP was raised ($p<0.01$). In three animals C_{rs} was decreased above $PEEP_{10}$.

C_p increased in all animals from $PEEP_2$ up to $PEEP_{10}$ ($p<0.01$, Fig. 9.6). The decrease in the last step from $PEEP_{10}$ to $PEEP_{12}$ was not significant.

Control values of C_{rs} at 3 hours after the last oleic acid injection ($n=3$) were similar to the values 2 hours earlier. The values of C_{rs} after the PEEP procedures were not different from control values at corresponding time after oleic acid administration.

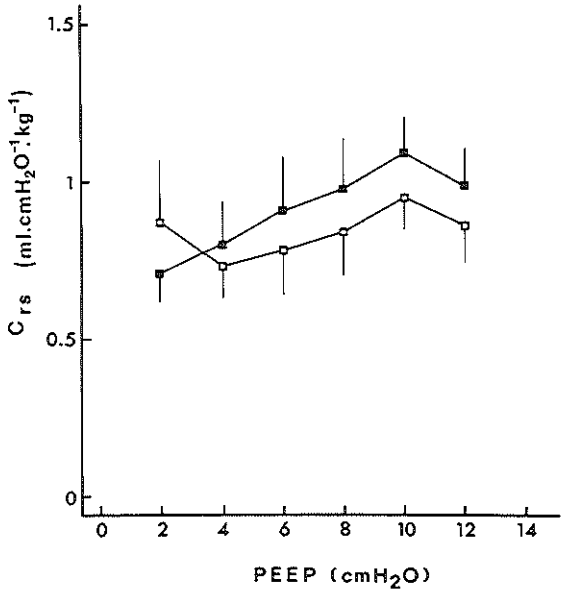


Fig. 9.6. Total respiratory compliance vs PEEP. For information see Fig. 9.2. Open squares: total respiratory compliance (C_{rs}) determined with inspiratory pause method, closed squares: total respiratory compliance (C_p) determined with one inspiratory pause (12 $ml.kg^{-1}$).

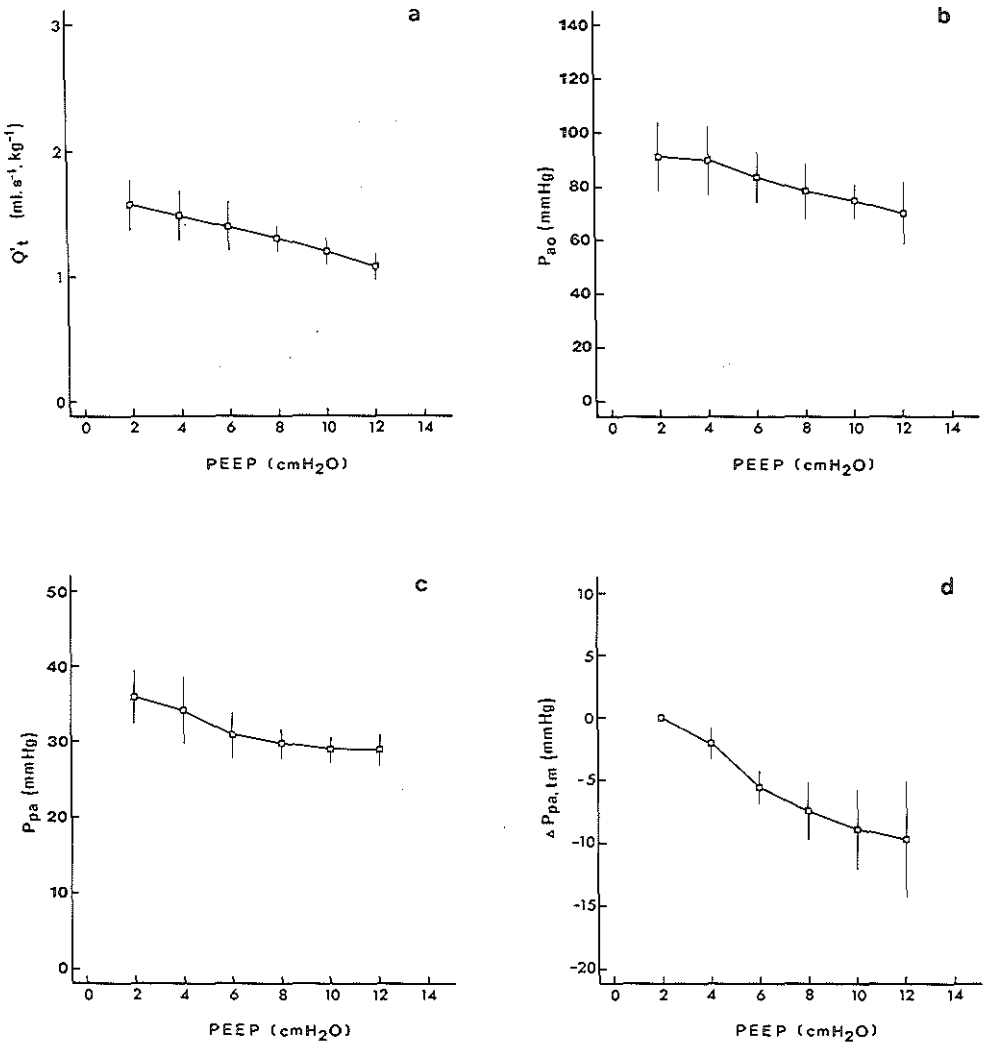
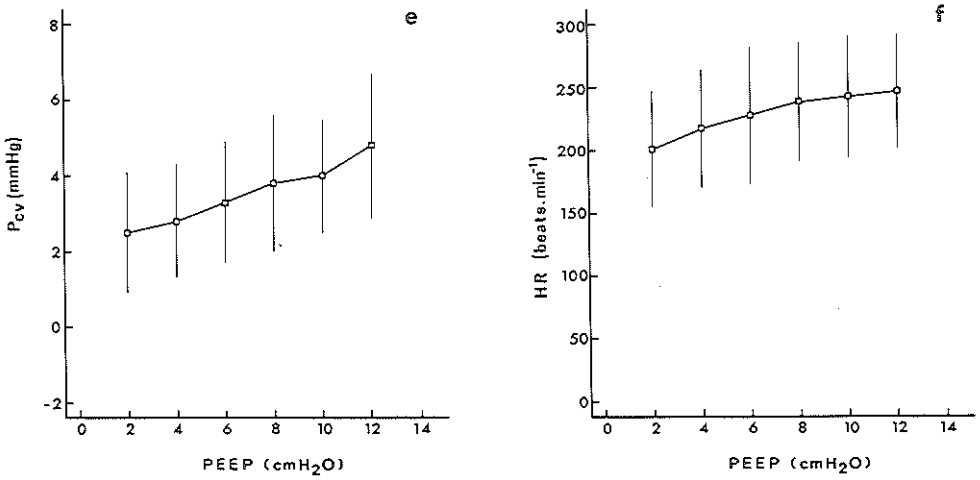


Fig. 9.7. Hemodynamic variables vs PEEP.
For information see Fig. 9.2. a. cardiac output, b. aortic pressure, c. pulmonary arterial pressure, d. changes in transmural pulmonary arterial pressure, e. central venous pressure, f. heart rate.



Hemodynamic data

Q'_t decreased linearly from 1.6 ± 0.2 ml.s^{-1}.kg^{-1} at $PEEP_2$ to 1.1 ± 0.1 ml.s^{-1}.kg^{-1} ($p=0.01$, Fig. 9.7.a) at $PEEP_{12}$. After return to $PEEP_2$, Q'_t was the same as before the PEEP procedures (Table 9.1).

P_{ao} decreased with each level of PEEP, from 91 ± 13 mmHg at PEEP 2 cmH_2O to 70 ± 11 mmHg at the highest level of PEEP. However, this decrease was just not statistically significant ($p=0.06$, Fig. 9.7.b). After the PEEP procedures P_{ao} remained low, but compared to its value before the PEEP procedures, it was not statistically different ($p=0.059$, Table 9.1).

P_{pa} decreased non-linearly from 36 ± 4 mmHg to 29 ± 2 mmHg with increasing PEEP ($p<0.01$ quadratic trend, Fig. 9.7.c) with the steepest fall between $PEEP_2$ and $PEEP_6$ and the slightest change above $PEEP_6$. $P_{pa,tm}$ decreased too with each step of PEEP ($p<0.01$, quadratic trend, Fig. 9.7.d), according to the same pattern as P_{pa}. When PEEP was reset to 2 cmH_2O, P_{pa} increased again and regained a value only sligthly lower than its value before the PEEP procedures ($p=0.04$, Table 9.1). However, no difference was found between P_{pa} after the PEEP procedures and its corresponding value of the control group.

P_{cv} increased linearly with the increase in PEEP, from 2.5 ± 1.6 mmHg at $PEEP_2$ to 4.8 ± 1.9 mmHg at $PEEP_{12}$ ($p<0.01$ Fig. 9.7.e). After the PEEP procedure, P_{cv} was 2.2 ± 1.4 ($n=5$), which was not different from its value before PEEP (Table 9.1).

Heart rate (HR) increased from 201 ± 46 beats/min at $PEEP_2$ to 246 ± 45

beats/min at $PEEP_{12}$ ($p<0.05$, linear trend, Fig. 9.7.f). After the PEEP procedures HR was not significantly different from before (Table 9.1).

R_{sys} did not change during the application of PEEP. However, after PEEP was reset to $PEEP_2$, R_{sys} was lower than its corresponding value before the stepwise rise of PEEP ($p<0.01$, Table 9.1).

Oxygen delivery

D_{O2} increased up to $PEEP_6$ and decreased at higher PEEP levels ($p<0.05$ quadratic trend, Fig. 9.8) After the PEEP procedures D_{O2} was the same as before (Table 9.1). The PEEP level where D_{O2} was maximal, was not the same in all animals. In 2 animals D_{O2} was maximal at 4 cmH_2O, in 3 animals at 6 cmH_2O and in 1 animal at 8 cmH_2O.

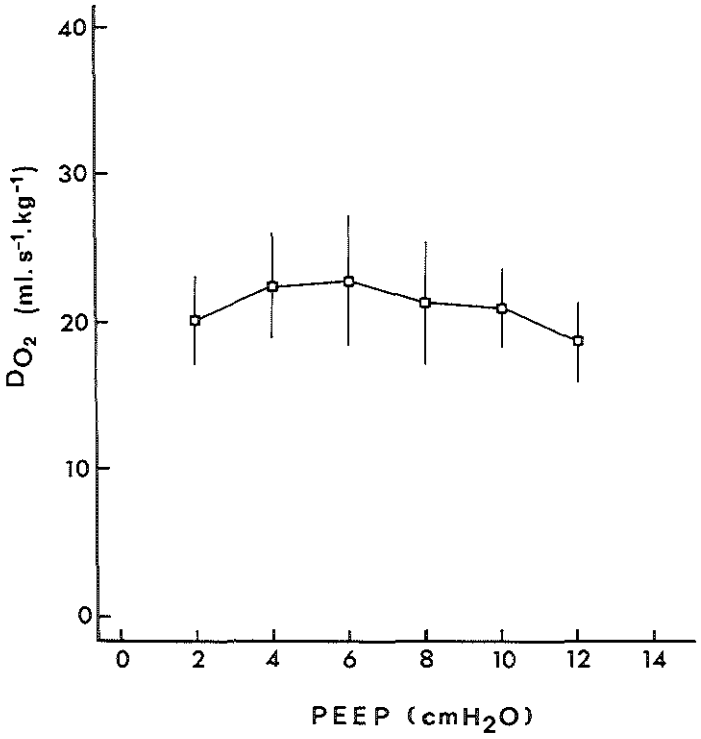


Fig. 9.8. Oxygen delivery. For information see Fig. 9.2.

Table 9.2. Prediction of maximal oxygen delivery by other variables

	-2 cmH_2O		"best PEEP"		+2 cmH_2O		+4 cmH_2O		+6 cmH_2O	
D_{O2}	22 ± 4	#	24 ± 4		22 ± 3	#	20 ± 3	#	19 ± 3	
P_{aO2}	79 ± 24	#	125 ± 54		144 ± 54		189 ± 61	#	211 ± 42	#
S_{aO2}	85 ± 8	*	95 ± 3		97 ± 2		98 ± 2	*	99 ± 0.7	
$P_{\bar{v}O2}$	34 ± 4	#	37 ± 5		36 ± 5		35 ± 6		35 ± 5	
$S_{\bar{v}O2}$	35.7 ± 7.6	#	41.2 ± 8.8		40.7 ± 8.6		38.9 ± 10.5		36.1 ± 5.3	
P_{aCO2}	65 ± 10		61 ± 11		59 ± 9		58 ± 9		58 ± 13	*
Q'_s/Q'_t	28 ± 11	#	15 ± 6		10 ± 4		7 ± 4	*	5 ± 2	*
V_D/V_T	58 ± 7	*	54 ± 7		53 ± 5		52 ± 5		51 ± 9	
Hb	7.3 ± 1.1		7.3 ± 1.2		7.3 ± 1.2		7.2 ± 1.1		7.3 ± 1.3	
pH	7.30 ± 0.06	*	7.32 + 0.07		7.32 ± 0.06		7.33 ± 0.06		7.31 ± 0.08	
P_{aCO2}-P_{etCO2}	23 ± 7	*	19 ± 6		16 ± 3		13 ± 3	*	12 ± 6	#
C_{rs}	0.7 ± 0.1		0.8 ± 0.1		0.8 ± 0.1		0.9 ± 0.1	*	0.9 ± 0.2	*
C_p	0.8 ± 0.1	#	0.9 ± 0.1		1.0 ± 0.2	*	1.0 ± 0.1	*	1.0 ± 0.2	*
V_{EE}	20 ± 6	#	26 ± 7		32 ± 7	#	40 ± 7	#	47 ± 5	#
Q'_t	1.5 ± 0.2		1.4 ± 0.2		1.3 ± 0.1	#	1.2 ± 0.1	#	1.1 ± 0.1	*
P_{ao}	90 ± 12		85 ± 10		79 ± 5	*	73 ± 7	*	68 ± 11	
P_{pa}	34 ± 3	#	31 ± 3		30 ± 2	#	29 ± 2	*	28 ± 2	
P_{cv}	2.7 ± 1.6	#	3.1 ± 1.6		3.8 ± 1.8	#	4.2 ± 1.7	#	4.8 ± 2.3	#
R_{sys}	59 ± 10		57 ± 9		58 ± 8		58 ± 8		58 ± 13	

"Best PEEP": PEEP at maximal oxygen delivery (D_{O2} in $ml.s^{-1}.kg^{-1}$). Mean values ± sd, n=6; at +6 cmH_2O n=4. For abbreviations and units see Table 9.1; C_p, compliance of pause procedure with volume of 12 $ml.kg^{-1}$. * $p<0.05$ and # $p<0.01$, compared to "best PEEP".

In Table 9.2 all other variables are presented with respect to the PEEP level at maximal D_{O2} and at levels lower and higher than this "best PEEP" level. From all variables $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ showed the same pattern of changes with PEEP as D_{O2}, i.e. an increase up to "best PEEP" and a decrease above "best PEEP". The other variables either increased, decreased or did not change with PEEP, from 2 cmH_2O below "best PEEP" up to 6 cmH_2O above "best PEEP". V_{EE} at "best PEEP" (Table 9.2) was not different from V_{EE} in baseline (Table 9.1), before oleic acid administration ($p=0.72$). V_{EE} at 2 cmH_2O above and below "best PEEP" was significantly different from V_{EE} at "best PEEP" and V_{EE} before oleic acid administration ($p<0.05$).

In Table 9.3, individual correlation coefficients of several selected variables with D_{O2} are given. In four animals a significant correlation existed between $P_{\bar{v}O2}$ and D_{O2}, in three animals between $S_{\bar{v}O2}$ and D_{O2}, whereas in one animal this latter correlation was just not significant ($p=0.06$). In two animals a correlation was found between P_{aO2} and D_{O2} and in the same two animals between V_{EE} and D_{O2}.

DISCUSSION

Stability of the oleic acid model

In a previous study (20), we presented a stable model of respiratory distress in pigs after multiple oleic acid injections of 0.1 ml. This model fulfilled the clinical criteria of ARDS (39,41).

Our present study was done with the same model. Baseline values were the same, except for V_{EE} (Table 9.1). We have no additional data to explain why V_{EE} in the present group was higher than in the previous group. After oleic acid administration, we found similar changes in gas exchange and hemodynamic variables compared with our previous study (Table 9.1). Also the reduction in V_{EE} was similar.

Half an hour after the PEEP procedures, the majority of variables had regained the initial $PEEP_2$ values (Table 9.1). P_{aO2} was slightly but not significantly higher than before the PEEP procedure. However, compared with the control group P_{aO2} was higher ($p<0.05$). Arterial oxygen saturation was just not statistically different from the control values. Therefore, when there is a rest effect of PEEP on P_{aO2}, it is only a slight one. After the PEEP procedure V_{EE} showed a large decrease again (Fig. 9.4 and Table 9.1), but remained slightly higher than before, corresponding with the value at $PEEP_4$. Perhaps this slight

Table 9.3. Correlation with D_{O2}.

EXP	P_{aO2}	$P_{\bar{v}O2}$	$S_{\bar{v}O2}$	Q'_s/Q'_t	C_{rs}	C_p	P_{aCO2}-P_{etCO2}	V_D/V_T	V_{EE}
1	r=-.60	r=0.95 *	r=0.99 **	r=0.11	r=-.42	r=-.53	r=-.15	r=-.13	r=-.61
2	r=-.95 *	r=0.58	r=0.61	r=0.72	r=-.46	r=-.52	r=0.81	r=0.72	r=-.91 *
3	r=-.57	r=-.17	r=-.29	r=0.45	r=-.50	r=-.47	r=0.77	r=0.64	r=-.80
4	r=0.30	r=0.95 *	r=0.99 **	r=-.36	r=0.53	r=0.57	r=-.16	r=-.54	r=-.02
5	r=-.92 *	r=0.95 **	r=0.85(p=0.06)	r=0.88	r=-.72	r=-.70	r=0.85	r=0.22	r=-.93 *
6	r=0.61	r=0.95 **	r=0.90 *	r=-.88 *	r=0.68	r=0.71	r=-.54	r=-.64	r=0.48
score	**n=2**	**n=4**	**n=3**	**n=1**	**n=0**	**n=0**	**n=0**	**n=0**	**n=2**

Individual correlation coefficients of variables (in headline) with oxygen delivery. EXP 1-6: individual experiments. For abbreviations and units see Table 9.1; for C_p, see Table 9.2. * $p<0.05$; ** $p<0.01$. In the last row the score of significant correlations is given for each variable.

rest effect on V_{EE} was the reason that the rest effect on P_{aO2} and S_{aO2} was not significant.

The hemodynamic variables before and after the PEEP procedures were not statistically different, except for R_{sys} and P_{pa}. Because cardiac output did not change, the decrease in R_{sys} coincided mainly with a fall in P_{ao}, although the difference between P_{ao} before and after the PEEP procedure were just not significant ($p=0.059$). The slight decrease in P_{pa} might be related to the slightly higher P_{aO2}. Probably some vasodilation of muscular pulmonary arteries occurred. pH, which did not change during the stepwise rise in PEEP, decreased significantly when PEEP was returned to $PEEP_2$. This might be due to a combination of the increase in P_{aCO2} and decrease in HCO_3^-, although these two changes in itself were not significant (Table 9.1).

It can be concluded that except for V_{EE}, R_{sys}, P_{pa} and pH, all conditions of the oleic acid model before the application of PEEP were regained, indicating that the effects of PEEP were temporary.

Effects of PEEP

After each stepwise increase in PEEP, a stabilisation period of 15 min was inserted, before measurements were started. It was demonstrated in patients with acute pulmonary failure, that this period is long enough to complete the total change in V_{EE} (30).

Gas exchange and pulmonary data

An increase in P_{aO2} by PEEP was also found by others in oleic acid induced respiratory distress (7,12,38,40,49). We attribute the increase in P_{aO2} to the increase in V_{EE} (Fig. 9.4), implying recruitment of alveoli (16,19,30). This recruitment leads to a decrease of area's with a low ventilation-perfusion (V'/Q') ratio and a decrease in Q'_s/Q'_t (Fig. 9.2.e), as was also seen in dogs with oleic acid induced injury (12,38) and ARDS patients (10,36). In one of the dog studies (38) it was demonstrated that PEEP reduced Q'_s/Q'_t not only by inflation of previously flooded and collapsed air spaces but also by redistribution of the excess of alveolar water into the compliant perivascular compartments. The total amount of extravascular lung water did not decrease with a PEEP of 10 cmH_2O in dogs with permeability edema caused by alloxan (23). This was also found in dogs with oleic acid edema that were treated with a PEEP of 20 cmH_2O (42).

A decrease in V_D/V_T and in Q'_s/Q'_t with a rise of PEEP up to $PEEP_{10}$ was also demonstrated in dogs with oleic acid induced lung injury and ascribed to a reduction of V'/Q' heterogeneity (7). At higher levels of PEEP (15 and 20 cm

H_2O) an increase in V_D/V_T was found, which the authors attributed to an increase of the anatomic dead space and higher V'/Q' ratio's. At $PEEP_{10}$ and $PEEP_{12}$ we found the first signs of such an increase. First, the change in V_D/V_T was flattened at these levels in the averaged values. Furthermore, in two animals a rise in V_D/V_T was observed between $PEEP_{10}$ and $PEEP_{12}$.

Another indication of the better V'/Q' ratio's during PEEP was the decrease in P_{aCO2}-P_{etCO2} (Fig. 9.3.b). The correlation between V_D/V_T and P_{aCO2}-P_{etCO2} was 0.86 and highly significant ($p << 0.001$). These results confirmed those of Murray et al. (40), who found a decrease in P_{aCO2}-P_{etCO2} in dogs, when PEEP was increased after oleic acid administration. Above $PEEP_{20}$ (i.e. 15 mmHg) they found an increase in the P_{aCO2}-P_{etCO2} gradient. Others did not find a change in P_{aCO2}-P_{etCO2} in patients with acute respiratory failure between zero PEEP, $PEEP_{9.3}$ and $PEEP_{20}$ (29). A reason for this difference could be a difference in stage of respiratory distress between the animals and the patients.

The increase in total respiratory compliance with PEEP is probably caused by a recruitment of previously collapsed alveoli (15,30,48), which is indicated in our results by the increase in V_{EE}. When recruitment is maximal, higher levels of PEEP may lead to overdistention and a decrease in C_{rs} (15,48), as we observed from $PEEP_{10}$ to $PEEP_{12}$. Dall'ava-Santucci et al. (8) did not find a change in total respiratory compliance with increasing PEEP in ARDS patients. They claimed that PEEP only changed end-expiratory pressure and volume, but not the pressure-volume (P-V) relationship, implying a shift of end-expiratory pressure and volume on this unchanged P-V relationship. In our pigs with oleic acid induced respiratory distress, PEEP actually shifted the P-V curve upwards (Fig. 9.5), indicating recruitment of alveoli. This recruitment implied a larger lung volume for the same pressure value on the P-V curves, if starting from a higher PEEP. We assume that this recruitment is a result of insufflation superimposed on end-expiratory lung volume. Collapse of alveoli presumably needs time, because after returning to $PEEP_2$ from $PEEP_{12}$ V_{EE} showed some rest effect.

Hemodynamic data

Cardiac output decreased during the application of PEEP after oleic acid administration, as was also found by many others in this model (6,37,40,43,47). The major cause of the decrease in Q'_t during PEEP is the decrease in venous return due to the increase in P_{cv} (Fig. 9.7.e) as a result of the increase in intrathoracic pressure (22,43,44). Additional mechanisms mentioned in literature are a compensation by baroreceptor reflexes and a negative effect on cardiac output by lung stretch receptor reflexes (44,45). We found a linear fall in cardiac output instead of a nonlinear fall observed by Schreuder et al. in the same species without any lung injury (44). A reason could be the involvement of an additional

mechanism in our experiments. The decrease in P_{aCO2} and the increase in P_{aO2} could have diminished sympathetic activity (34) and therefore might have contributed to the decrease in cardiac output. We have no information whether lungs with oleic acid injury behave differently from normal lungs with respect to the lung stretch reflex as a mechanism for the nonlinear fall in cardiac output with PEEP (44).

We did not calculate pulmonary vascular resistance based on only the Poiseuille type of flow resistance using the hemodynamic equivalent of Ohm's law ($\Delta P/Q'=R$), because during mechanical ventilation two types of flow resistance will exist, being Poiseuille resistance and Starling resistance (50). Therefore, we had to conclude from changes in pressure and flow whether the fall in P_{pa} could be attributed to either the fall in cardiac output or vasodilation of muscular pulmonary arteries or both. Unfortunately, we had no data of pulmonary capillary pressure to calculate pulmonary arterial vascular resistance. We regarded the formula of Gaar et al. (17) not applicable because the ratio between pulmonary arterial vascular resistance and pulmonary venous vascular resistance will have been changed by vasoconstriction and vessel occlusion after oleic acid administration.

The effect of PEEP on pulmonary vascular resistance is doubtful. In pigs with normal lungs we usually observed an intrathoracic pressure between -2 mmHg and -4 mmHg (51). After oleic acid administration lung volume was lower, therefore we assumed an averaged P_{it} value of -4 mmHg. This implied a $P_{pa,tm}$ of about 40 mmHg, equal to P_{pa}(36 mmHg)-P_{it}.

$P_{pa,tm}$ decreased about 10 mmHg when PEEP was raised up to $PEEP_{12}$ (Fig. 9.7.d), resulting in a $P_{pa,tm}$ at highest PEEP of 30 mmHg, which does not indicate a return to normal pulmonary vascular resistance. This level is surprisingly high because P_{aO2} at $PEEP_{12}$ is on average 226 mmHg, which does not imply alveolar hypoxia. Apparently the high level of $P_{pa,tm}$ was due to other mechanisms: 1) compression of alveolar vessels as a result of and increased alveolar pressure by $PEEP_{12}$ and the superimposed insufflation pressure; 2) vasoconstriction induced by mediators from granulocytes (20); and 3) intravascular obstruction by clotting (21).

Indicators of maximal oxygen transport

Up to "best PEEP" the increase in arterial oxygen content prevailed over the negative effect on cardiac output. Above "best PEEP" the decrease in cardiac output became more dominant and caused the decline in D_{O2}. In another oleic acid study with dogs maximal D_{O2} was found at zero end-expiratory pressure and

D_{O2} decreased gradually with a rise in PEEP (40). The reason was probably that the respiratory distress was moderate, characterized by an averaged P_{aO2} of about 110 mmHg at an F_{IO2} of 0.5. A similar effect of PEEP was also found in pigs with normal lungs (44), where oxygen saturation was constant and cardiac output decreased with PEEP.

In our pigs with an early respiratory distress, "best PEEP" was not predicted by total respiratory compliance. In only three pigs C_{rs} decreased slightly above $PEEP_{10}$, indicating the presence of a maximum. In Suter's study, the PEEP level with maximal total respiratory compliance, called optimal PEEP, was slightly higher than that with maximal oxygen transport. This was also found by others in ARDS patients with high venous admixture (35). However, in patients with very low values of total respiratory compliance (15) or in the late fibrotic stage of acute pulmonary failure (33), compliance was not a useful indicator for "best PEEP". The discrepancies between these patient studies might be due to differences in stages of the respiratory distress.

P_{aCO2}-P_{etCO2} was not a good indicator of "best PEEP" in our study. Above the level of "best PEEP", P_{aCO2}-P_{etCO2} was still decreasing and no individual correlation was found with D_{O2}. Although P_{aCO2}-P_{etCO2} is not a good indicator of maximal oxygen delivery, we agree with Murray et al (40) that it is a useful indicator of optimal V'/Q' ratio's.

A remarkable observation was that V_{EE} at "best PEEP" was similar to the value at $PEEP_2$ before oleic acid administration. Reference values of V_{EE} at $PEEP_2$ in normal individuals are not known. Perhaps reference values of FRC might be a substitute. This, however, will need clinical evaluation.

From the next two equations it can be understood, why we found a correlation of $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ with D_{O2}:

$$D_{O2} = C_{aO2} \times Q'_t \quad (1)$$

$$V'_{O2} = (C_{aO2}-C_{\bar{v}O2}) \times Q'_t \quad (2)$$

V'_{O2} was constant during the PEEP procedures. From $PEEP_2$ to "best PEEP" D_{O2} was increased due to a rise in C_{aO2} and a relatively smaller decrease in Q'_t. If C_{aO2} increases more than Q'_t decreases, then a rise in $C_{\bar{v}O2}$ occurs according to equation (2). From "best PEEP" to higher PEEP D_{O2} decreased due to a decrease in Q'_t, whereas C_{aO2} hardly increased. This implies a fall in $C_{\bar{v}O2}$ according to equation (2). Thus, $C_{\bar{v}O2}$ is increased up to "best PEEP" and decreased above "best PEEP", as is true for D_{O2}. Suter et al. also found in patients with acute respiratory failure that maximal $P_{\bar{v}O2}$ coincided with maximal D_{O2} (48).

A condition for $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ as indicators of "best PEEP" is a constant oxygen uptake. In ARDS and septic patients it has been shown that oxygen uptake was dependent on oxygen supply (9,46). If V'_{O2} increases with D'_{O2}, it cannot be predicted from equation (2), whether and in which direction $C_{\bar{v}O2}$ will be changed.

Conclusions

In all animals with oleic acid induced respiratory distress, the application of PEEP increased oxygen delivery up to a maximum. The level of PEEP at maximal oxygen delivery was different for the different animals. This maximum was not predicted by total respiratory compliance, P_{aCO2}-P_{etCO2}, V_D/V_T or Q'_s/Q'_t. At "best PEEP" end-expiratory lung volume was equal to its baseline value before oleic acid administration. In four of the six animals D_{O2} correlated significantly with $P_{\bar{v}O2}$ and in half of the animals with $S_{\bar{v}O2}$, which implies that these variables might be indicators for determination of the appropriate level of PEEP if oxygen uptake is constant. Nevertheless, the best indicator of "best PEEP" is D_{O2}.

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CHAPTER X

COMPARISON OF THE LAVAGE AND OLEIC ACIC MODEL

The development of the two experimental models of respiratory distress, obtained by lavage and oleic acid, has given us the possibility to study many problems under standardized conditions. In the lavage model atelectasis and increased vascular permeability are induced by removal of surfactant (8, Chapter III), whereas oleic acid acts on the other side of the alveolar capillary membrane via deleterious effects on the capillary wall (2,9,13 and Chapter VII).

In this chapter we compared the results obtained in both models. Moreover some directions for future research will be discussed.

Conditions

Female pigs of about 10 kg body weight were used in all studies on the lavage and oleic acid model. There were no statistical differences in body weight between the pigs of the different studies. Anesthesia and ventilatory settings were similar and the same surgical procedures were performed in all animals. A detailed description of these surgical procedures can be found in each specific chapter. Also the methods of data aquisition and data analysis were the same.

Comparing the protocols of lavage and oleic acid administration, there were differences in time span between both models. In the lavage model a severe hypoxemia was established by a standardized regime of number and time sequence of lavages, two lavages at five minutes interval followed by another two lavages after 1 hour. In the oleic acid model, however, the number and time sequence of injections had to be adapted individually to obtain a comparable hypoxemia in all animals. An illustration of the difference between the standardized regime in the lavage group and the individually adapted regime in the oleic acid group, is the smaller spread of P_{aO2} and arterial oxygen saturation (S_{aO2}) in the latter group.

Another difference was an infusion of 10 $ml.kg^{-1}$ dextran into the pigs before the oleic acid administration. In the lavage model such infusion was not given, but the unrecovered lavage fluid, in total on average 25 $ml.kg^{-1}$, implied a larger fluid load than the dextran infusion in the oleic acid treated animals.

Table 10.1. Comparison of lavage and oleic acid model.

		baseline	60 min	120 min	240 min
P_{aO2} (mmHg)	L	308 ± 15	61 ± 29	55 ± 14	53 ± 10
	O	292 ± 27	55 ± 6	55 ± 2	52 ± 4
S_{aO2} (%)	L	99.8 ± 0.3	63.3 ± 24.6	65.5 ± 22.5	61.5 ± 16.3
	O	99.2 ± 1.1	72.0 ± 7.5	67.7 ± 8.7	65.2 ± 13.4
P_{aCO2} (mmHg)	L	40 ± 2	63 ± 9	64 ± 9	71 ± 6
	O	41 ± 3	58 ± 9	61 ± 10	67 ± 14
Q'_s/Q'_t (%)	L	4 ± 2	52 ± 24	54 ± 21	56 ± 13
	O	7 ± 2	43 ± 6	46 ± 9	43 ± 9 (n=3)
V_D/V_T (%)	L	34 ± 4	56 ± 5	56 ± 5	61 ± 3
	O	34 ± 11	58 ± 3	56 ± 6	53 ± 3 (n=3)
Hb ($mmol.l^{-1}$)	L	5.8 ± 0.8	6.6 ± 1.5	6.8 ± 1.4	7.6 ± 0.7
	O	6.5 ± 0.5	7.7 ± 1.2	8.0 ± 1.0	8.1 ± 1.2
Q'_t ($ml.s^{-1}.kg^{-1}$)	L	1.9 ± 0.2	2.4 ± 0.8	2.3 ± 0.5	2.3 ± 0.5
	O	2.1 ± 0.3	1.4 ± 0.3 *	1.5 ± 0.2 *	1.4 ± 0.2 *
P_{pa} (mmHg)	L	11 ± 4	29 ± 6	30 ± 7	30 ± 6
	O	15 ± 2	38 ± 2 **	37 ± 3 **	34 ± 3 *
P_{cv} (mmHg)	L	-0.1 ± 1.5	0.7 ± 2.3	0.2 ± 1.7	-0.4 ± 1.7
	O	1.5 ± 0.8	3.5 ± 1.4 *	3.0 ± 1.9 *	2.9 ± 2.0 *
D_{O2} ($ml.s^{-1}.kg^{-1}$)	L	28 ± 6	20 ± 4	22 ± 5	24 ± 7
	O	33 ± 4	18 ± 1	18 ± 2	18 ± 6

L, lavage group; O, oleic acid group. Mean values ± 1 sd; n=6, at 240 min oleic acid group n=5. Some measurements failed because of technical reasons, as indicated in the table. * $p<0.05$, ** $p<0.01$. For abbreviations, see text.

Comparison of the lavage and oleic acid model consists of three parts :

- comparison of physiological variables of gas exchange and circulation during the phase of stationary hypoxemia and standard mechanical ventilation;
- comparison of the morphologic findings in these models;
- comparison of the effects of PEEP in both models.

For statistical testing we used standard repeated measures analysis of variance (SPSS-Manova) and the unpaired Student t-test.

Common features of the models

In both models a severe hypoxemia was induced, P_{aO2} of about 50-60 mmHg at an F_{IO2} of 0.6, which was stable for 4-6 hours (Table 10.1). Both models fulfilled criteria of ARDS by Petty (11) and Murray et al. (10).

Calculation of the lung injury score (10) revealed no differences in degree of injury. In the lavage animals this score was on average 2.5, in the oleic acid group 2.4.

Comparable changes in other gas exchange variables like venous admixture (Q'_s/Q'_t), P_{aCO2}, and physiological dead space (V_D/V_T) were seen in the first four hours of stationary hypoxemia (Table 10.1). In the fifth and sixth hour a distinct deterioration of these variables occurred in the lavage animals (Chapter III).

The comparable disturbances in gas exchange as well as the similar decrease in end-expiratory lung volume (V_{EE}, Table 10.2) in both models can be attributed to the formation of edema and atelectasis.

The formation of edema in both models was illustrated by several factors:

- bilateral diffuse infiltrations on chest X-rays,
- increased weight of heart and lungs,
- increase in hemoglobine (Hb) concentration, indicating extravasation of fluid.
- thickening of septa implying the presence of interstitial edema.

In both models signs of acute inflammation of the lungs were observed, as a bronchopneumonia in all lobes, sometimes accompanied by an infiltration of the interstitium with granulocytes. These lesions varied from mild and focal to severe and diffuse. We suppose that the accumulated granulocytes in the lungs have been

Table 10.2. Pulmonary data.

		before lung injury n=6	after lung injury n=6
V_{EE} (ml.kg^{-1})	L	23.0 ± 2.5	13.7 ± 2.1
	O	21.0 ± 2.6	10.9 ± 2.9 (n=5)
C_{rs} (ml.cmH_2O^{-1}.kg^{-1})	L	1.7 ± 0.2	0.6 ± 0.1
	O	1.8 ± 0.3	0.9 ± 0.3 (n=5) *

Comparison of lavage (L) and oleic acid (O) group. Mean values ± 1 sd. Some measurements failed due to technical reasons. V_{EE}, end-expiratory lung volume, C_{rs}, total respiratory compliance. * $p<0.05$, compared to lavage group.

involved in the pathogenesis of the observed lung injury and increase of membrane permeability after lavage. In rabbits that were made leucopenic, lavages resulted in a less severe hypoxemia and albumin leakage into the lungs (6), compared with untreated animals. Another factor involved in the induction of increased membrane permeability is probably surfactant depletion caused by the lavages (1).

After oleic acid administration, granulocytes did not seem to be of primary importance for initiation of lung injury (4,5). It was demonstrated that oleic acid had a direct toxic effect on the endothelial cells (4,7,13). This does not exclude, however, that granulocytes contribute to the injury after the primary action of oleic acid.

Differences between the models

The weight of heart and lungs was 33.2 ± 4.1 g.kg^{-1} in the oleic acid group and 27.2 ± 2.2 g.kg^{-1} in the lavage group ($p<0.05$), indicating that lung edema was more severe in the oleic acid treated animals.

Because of the larger amount of edema in the oleic acid treated animals, one would expect a larger decrease in total respiratory compliance (C_{rs}) in this group, as compared with the lavage group. However, C_{rs} was slightly more decreased in the lavage animals (Table 10.2, $p<0.05$). We attibute this larger decrease, in spite of less edema, to a depletion of surfactant. In a representative animal of this

group, we observed signs of surfactant depletion as a pronounced reduction of lamellar bodies in pneumocytes II (Fig. 3.7). In the animals that received oleic acid no signs of such depletion were observed as a normal amount of lamellar bodies was present in the pneumocytes II (Fig. 7.12). This does not exclude, however, that surfactant function was attenuated by edema in these animals, as was demonstrated by Hall et al. (3), albeit to a lesser extent than the decrease in surfactant function of the lavage group.

The most striking differences between the two models were found in the changes of hemodynamic variables. In the lavage model there was no indication of any depressive effect on the heart and circulation. Cardiac output (Q'_t) and central venous pressure (P_{cv}) were not changed after the lavages. In the oleic acid model, however, P_{cv} was increased and Q'_t was decreased considerably after the last oleic acid injection (Table 10.1). Both variables were significantly different between the two models (Table 10.1, $p<0.05$). Pulmonary arterial pressure (P_{pa}) was much higher in the oleic acid group than in the lavage group (Table 10.1, $p<0.01$). Comparison of medial thickness of muscular pulmonary arteries in corresponding regions of the lungs did not reveal any statistical differences between the lavage and oleic acid group, implying a similar degree of active vasoconstriction of muscular pulmonary arteries. As P_{pa} was higher in the oleic acid group, we suppose that other factors than active vasoconstriction alone were involved in the development of pulmonary hypertension. A distinct histologic feature of the oleic acid model, was the frequent occurrence of vascular lesions, whereas in the lavage model these lesions were hardly seen. These vascular lesions caused by oleic acid consisted of extensive vasculitis of both arteries and venes (Fig. 7.10), fibrinoid necrosis and fibrine thrombi in some arteries (Fig. 8.1), and swelling of endothelial cells with signs of degeneration, like vacuolisation (Fig. 7.11). In Chapter VIII we concluded that all these vascular lesions might contribute to the development of pulmonary hypertension after oleic acid administration. Another factor that might have caused the higher P_{pa} after oleic acid administration, could be compression of vessels by the larger amount of edema in this group as compared to the lavage group.

Because ventilatory conditions were the same in both groups and because we had no indication for any differences in thoracic compliance between the groups, we assume that similar values of intrathoracic pressure existed, implying a higher transmural P_{cv} ($P_{cv,tm}$) and a higher transmural P_{pa} ($P_{pa,tm}$), i.e. afterload of the right ventricle, in the oleic acid group compared to the lavage group.

Calculation of right ventricle power ($W_{rv}=Q'_t \times P_{pa,tm}$) revealed no differences between both models ($p=0.30$ at 60 min after the last lavage or oleic

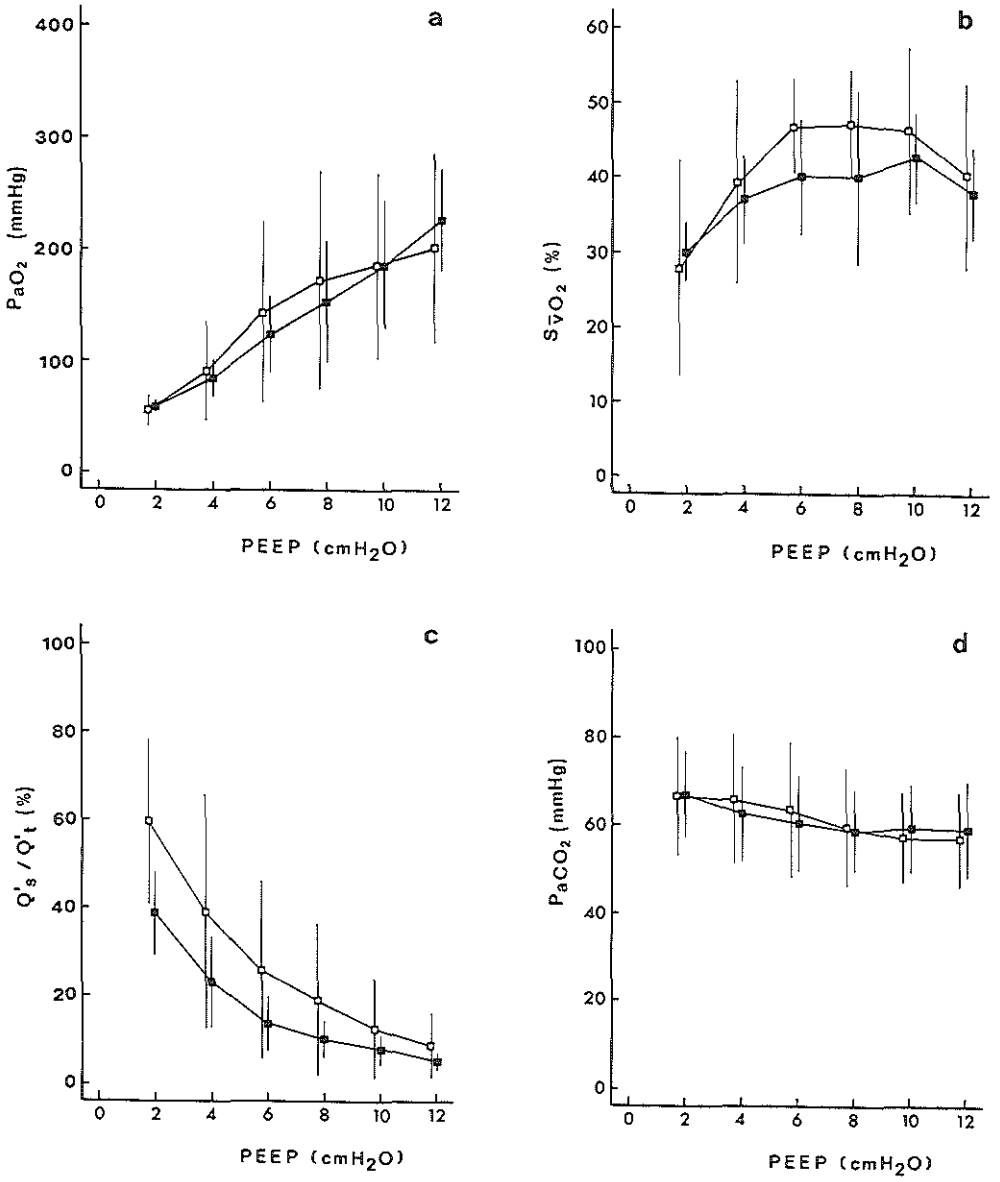
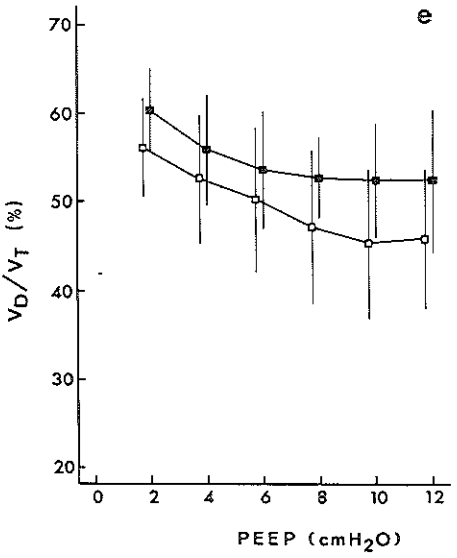


Fig. 10.1. Gas exchange variables vs PEEP.

Variables of gas exchange of lavage and oleic acid model plotted against positive end-expiratory pressure. Open squares: lavage group, closed squares: oleic acid group. Mean values ± 1 sd (vertical bars), n=6, at $PEEP_{12}$ n=5 in oleic acid group. There were no statistical differences in mean value or slope between the two models. a. arterial oxygen tension; b. mixed venous oxygen saturation, c. venous admixture; d. arterial carbon dioxide tension, e. physiological dead space.



acid injection). We have calculated ventricular power, i.e. work per time unit, instead of work per stroke in order to compensate for differences in beat cycle time. Thus, for a similar right ventricular power, a higher $P_{cv,tm}$ was found in the oleic acid group as compared to the lavage group ($p<0.05$). This is an indication that heart function is attenuated in the oleic acid treated animals. Schuster et al. (14) have shown in dogs a decrease in myocardial contractility after oleic acid injection. However, we cannot exclude, that at a higher afterload a higher filling pressure is needed to deliver the same power.

The lower cardiac output in the oleic acid group as compared to the lavage animals, combined with a similar degree of hypoxemia did not lead to a significantly larger decrease in oxygen delivery (D_{O2}, $p=0.16$ Table 10.1). This might be due to a combined effect of two non-significant differences between both groups: the slightly higher Hb concentration and arterial oxygen saturation in the oleic acid group.

Conclusions

Comparable disturbances in gas exchange and lung mechanics could be induced in all animals with both methods, lavage and oleic acid administration. Clinical criteria for severe lung injury were approximated (10,11).

In contrast with the lavage model, functions of the circulatory system in the oleic acid model were compromized, which was probably the reason for death of two animals in this group at 3.5 and 4 hours after the last oleic acid injection. Nevertheless, mortality in both models was low in comparison with data presented in literature and a satisfactory period of respiratory distress of 4-6 hours could be obtained. Such a stable period makes study of interventions possible.

Both models showed clear signs of an acute inflammation of the lungs. Moreover, in the oleic acid model vascular lesions were prominent, whereas in the lavage model signs of depletion of lamellar bodies in the pneumocytes II were observed.

Comparison of PEEP effects

In our PEEP studies with the lavage (Chapter V) and oleic acid model (Chapter IX), similar conditions of respiratory distress were obtained as in our first studies on the models under standard mechanical ventilation, indicating a good reproducibility of each of the models.

The effects of PEEP on gas exchange variables in both the lavage and oleic acid model were not statistically different (Fig. 10.1). This also was true for the effects on hemodynamic variables (Fig. 10.2), although the heart worked at a higher filling pressure after oleic acid administration, implying a higher P_{pa} and P_{cv}, and lower Q'_t at the start of the PEEP procedures.

In all animals of both the lavage and oleic acid group a maximum in D_{O2} was found when PEEP was applied. Although the level of PEEP where D_{O2} was maximal ("best PEEP") showed some individual differences, the similarity of the mean curves of both groups is striking (Fig. 10.3).

Apparantly, the method used to induce respiratory distress, lavage or oleic acid, is not crucial for studying PEEP effects in a respiratory distress model, as long as a comparable respiratory distress is present.

Indicators of "best PEEP"

In both models we evaluated which variables could be useful as an indicator of "best PEEP". None of the indicators mentioned in the literature, i.e. total respiratory compliance, arterial minus end-tidal P_{CO2} gradient, physiological dead space and venous admixture appeared to be reliable indicators of "best PEEP".

Mixed venous P_{O2} and S_{O2} had the best correlation with D_{O2} in both models

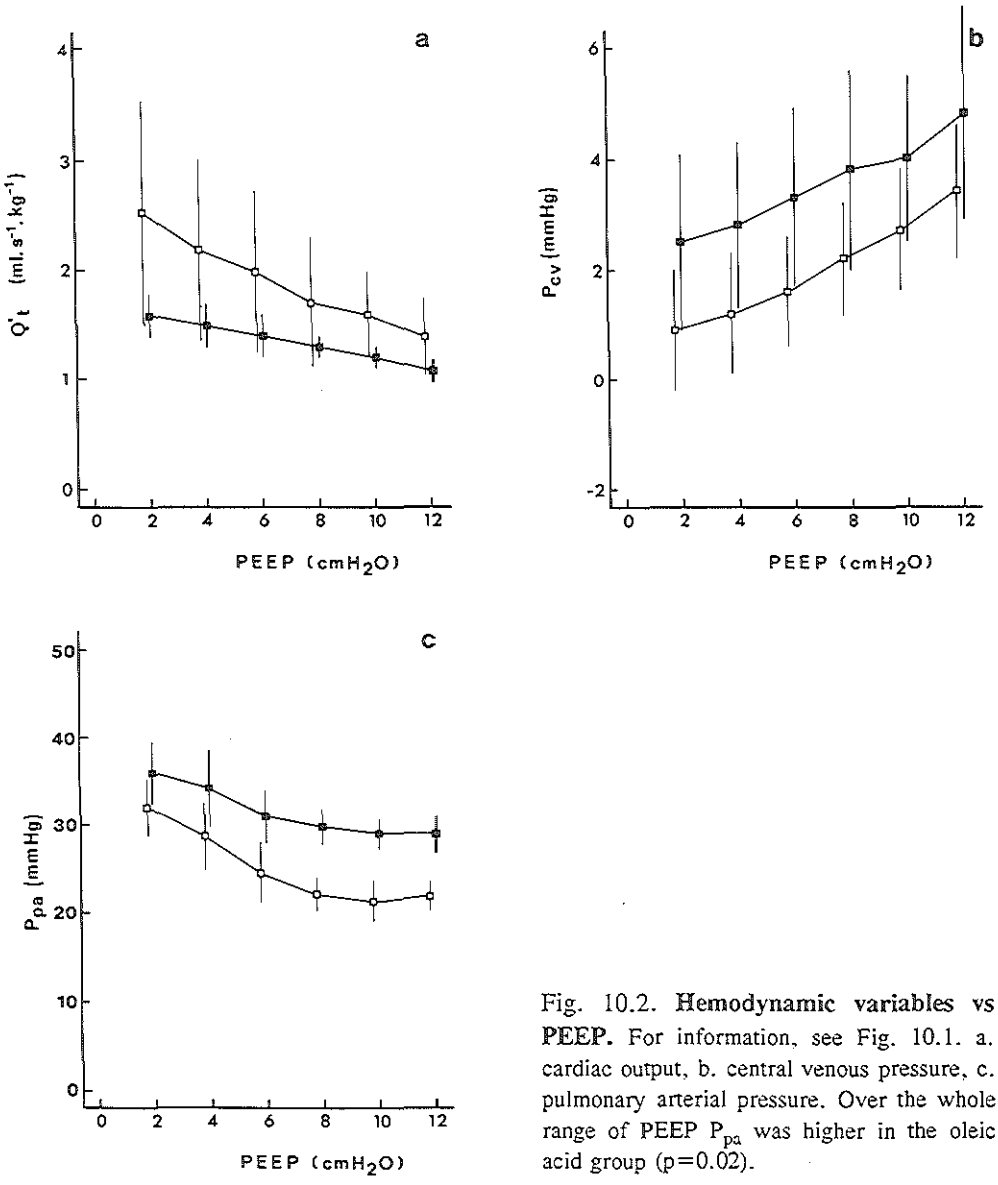


Fig. 10.2. **Hemodynamic variables vs PEEP.** For information, see Fig. 10.1. a. cardiac output, b. central venous pressure, c. pulmonary arterial pressure. Over the whole range of PEEP P_{pa} was higher in the oleic acid group ($p=0.02$).

(Tables 5.3 and 9.3). In the lavage model these variables correlated significantly in 5 out of 6 animals and in the oleic acid model in 4 out of 6. Therefore, these variables could be useful as predictors of "best PEEP".

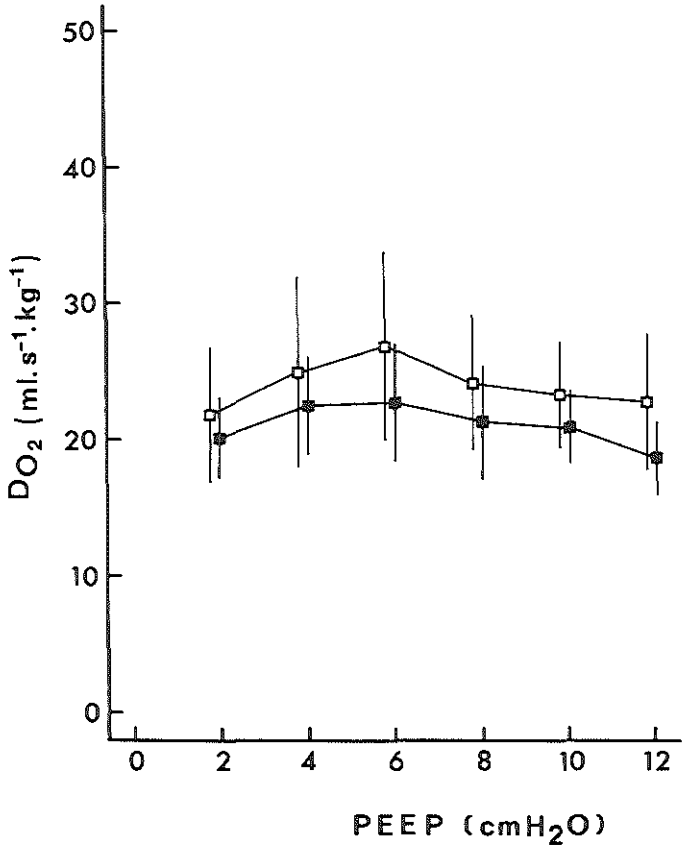


Fig. 10.3. Oxygen delivery.
For information, see Fig. 10.1.

Directions for future research

The development of two models of respiratory distress, both stable for at least 4 hours, provides possibilities to study interventions of all kind. Because both models were developed under more or less the same conditions comparison of results of such interventions may give more insight in the understanding of pathophysiological mechanisms.

As in both models severe inflammation of the lungs is induced, it would be interesting to investigate whether pharmacological intervention with e.g. anti-inflammatory drugs or oxygen radical scavengers could prevent or cure this

inflammatory proces, and whether such interventions have the same result independent of the origin of the respiratory distress.

Because most variables were stable for at least four hours in both models, we regard these models suitable for studying the effects of different ventilatory strategies.

In normal lungs, mechanical ventilation caused a shift of blood from the pulmonary to the systemic circulation during insufflation and vice versa during expiration (15). This shift ia larger when tidal volume is larger. Although mechanical ventilation has a overall positive effect on gas exchange, this shift in blood volume is in itself a negative effect. Morphologic investigation of the lungs in our two models of respiratory distress revealed a inhomogeneously distributed pulmonary vasoconstriction in all lobes, which was probably related to local differences in ventilation due to edema or atelectasis. Assuming that the more compliant "healthy" parts of the lungs receive most of the tidal volume during mechanical ventilation, it is likely that more blood will be shifted away from these areas, including the lung capillaries, than from the diseased parts. This decrease in lung capillary blood volume will have a negative effect on gas exchange (12) The models that we described in this thesis could be used to test this hypothesis.

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CHAPTER XI

SUMMARY

Chapter I

The respiratory distress syndrome is characterized by an acute decrease in arterial oxygen tension and respiratory system compliance without a history of chronic lung disease or heart failure. Respiratory distress can arise from several causes. Two main syndromes of respiratory distress are known: adult respiratory distress syndrome (ARDS) and infant respiratory distress syndrome (IRDS). Application of positive end-expiratory pressure (PEEP) has positive effects on gas exchange and negative effects on the circulation. In this chapter features of ARDS and IRDS and different approaches of PEEP application are described.

Animal models of respiratory distress can be used to study basic mechanisms and therapy. So far, most existing animal models have a lack of long lasting stability. Hardly any attention was paid to standardization of the induction of respiratory distress. In this thesis two models of respiratory distress were studied. The first model was induced with alveolar lavage as an analogy of primary depletion of surfactant, acting on the epithelial side of the alveolo-capillary membrane. The second model was obtained by intravenous administration of oleic acid as an analogy for fat embolism, acting on the endothelial side.

Chapter II

Impairment of lung surfactant is involved in the pathogenesis of ARDS and IRDS. Near-drowning as an origin of ARDS is most markedly associated with surfactant deficiency. Tension of air-water tends to reduce the area of the interface. Surfactant decreases this surface tension and keeps the alveoli open. Surfactant deficiency mainly results in atelectasis. Total lung lavages in animals were performed to study effects of surfactant depletion. In studies on lavage models, ventilatory settings were often changed and practically no data were available to evaluate the stability of the animal's condition after lavage. "Best PEEP", which is defined as the level of PEEP where oxygen delivery is maximal, has not been studied after lavage. We aimed to develop a model of respiratory distress by lavages in pigs with volume controlled ventilation and an inspiratory

oxygen fraction of 0.6. This model should fulfil clinical criteria, should have a low mortality and should be stable for several hours to study interventions. As an intervention we additionally studied the effects of PEEP to find indicators of "best PEEP".

Chapter III

We aimed at a regime of lung lavages to induce a respiratory distress in pigs. Twenty-three pigs of 9.6 ± 0.8 (sd) kg body weight were anesthetized and paralyzed with continuous infusions of sodium pentobarbital and tubocurarine respectively. Additional conditions were volume controlled mechanical ventilation, a PEEP of 2 cmH_2O and an F_{IO2} of 0.6. Each lavage was performed with 35 $ml.kg^{-1}$ of 0.9% saline. Comparing different regimes of lavages we hypothesized that surfactant was maximally released from the pneumocytes II within one hour after a first pair of lavages. After the experiments we observed hardly any lamellar bodies in the pneumocytes II.

The best regime consisted of a pair of lavages within five minutes, followed after one hour by another pair of lavages. This regime caused an arterial oxygen tension of 47.8 ± 11.9 mmHg (sd, n=8). Cardiac output, aortic pressure and central venous pressure were similar to baseline values. Oxygen delivery was lower and pulmonary arterial pressure higher than baseline values. These variables were stable during 5 h. We recommended a regime of two pairs of lavages at an interval of one hour for studies on early respiratory distress.

Chapter IV

Severity and distribution of active vasoconstriction in early respiratory distres were studied by assessing pulmonary arterial medial thickness. A respiratory distress, stable for 6 hours, was induced in mechanically ventilated pigs by lung lavages with saline to wash out surfactant. Oxygen tension in arterial blood was 53 ± 13 mmHg (at an inspiratory oxygen fraction of 0.6) and mean pulmonary arterial pressure was 31 ± 6 mmHg. In a control group lavages were simulated by interruptions of the ventilation for the same duration as lavages with saline. The animals were killed with sodium pentobarbital. As a measure of vasoconstriction, medial thickness as percentage of external diameter of muscular pulmonary arteries was determined in various parts of the lung. In lavage induced respiratory distress, medial thickness was significantly increased as compared to controls (7.7 ± 2.9 % and 3.7 ± 1.5 % respectively), indicating severe

vasoconstriction. The severity of vasoconstriction was on average the same in the different lung regions. Vasoconstriction varied considerably within each region and was even absent in occasional vessels. Constriction was attributed to local hypoxia and mediators from granulocytes.

Chapter V

In the animal model of early respiratory distress as described in Chapter III, we studied the effects of positive end-expiratory pressure (PEEP) to investigate indicators of "best PEEP". We used a group of six young pigs with a body weight of 9.5 ± 0.4 (sd) kg. The gas exchange and hemodynamic data after lavage were similar to those of the group in Chapter III.

PEEP was increased from $PEEP_2$ (PEEP of 2 cmH_2O) by steps of 2 cmH_2O up to $PEEP_{14}$. Each step was maintained for half an hour. The following effects were obtained by increasing PEEP from $PEEP_2$ to $PEEP_{14}$: P_{aO2} increased from 55 ± 14 mmHg to 218 ± 94 mmHg and V_{EE} from 12 ± 2 $ml.kg^{-1}$ to 46 ± 9 $ml.kg^{-1}$; P_{aCO2} decreased from 66 ± 13 mmHg to 53 ± 9 mmHg and cardiac output from 2.5 ± 1.0 $ml.s^{-1}.kg^{-1}$ to 1.2 ± 0.3 $ml.s^{-1}.kg^{-1}$; central venous pressure increased from 0.9 ± 1.1 mmHg to 4.1 ± 1.4 mmHg. Oxygen uptake was not changed by PEEP. Oxygen delivery and $P_{\bar{v}O2}$ increased until about $PEEP_6$ and decreased at higher PEEP levels. The PEEP level at maximal D_{O2}, "best PEEP", was at about 6 cmH_2O. $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ correlated well with D_{O2} in five and four animals respectively. All other variables as P_{aCO2}-P_{etCO2}, physiological dead space, C_{dyn} and venous admixture did not correlate with D_{O2} and were regarded to be unreliable predictors of "best PEEP".

Chapter VI

Clinical aspects and pathogenesis of the fat embolism syndrome are discussed. Attention is focussed on the origin of fat after trauma, the contribution of lipase which can hydrolyse neutral fat to fatty acids like oleic acid, and the involvement of the complement system and extrinsic clotting cascade.

Since the first case of traumatic fat embolism was clinically recognized in 1873 by Bergmann, experimental studies have been performed. Comparison of effects of neutral fat injection and injection of fatty acids in animals revealed that oleic acid was a far more toxic substance than neutral fat, indicated by a lower minimal lethal dose. It was demonstrated that oleic acid had a direct toxic effect on the endothelial wall. Inhibition of the Ca^{2+} pump and (Na^{+}-K^{+})-ATPase

leads to dysfunction of the cell membrane. Accumulated platelets and leukocytes in the lungs after oleic acid administration may contribute to the injury after the primary insult of oleic acid. In 1968 injection of oleic acid in dogs was presented as a method to induce clinical and pathological features of respiratory distress after fat embolism. Thereafter, oleic acid has been used by many authors in different doses and in different animals. In most studies, of which three were performed in pigs, only a mild respiratory distress was obtained and a large individual spread in P_{aO2} was seen after a single dose. Mortality was high up to 30 %. The main objectives of our studies in pigs with oleic acid were: 1) to induce an animal model of severe respiratory distress that fulfilled clinical criteria, which was characterized by a low mortality and a stability for several hours, 2) to describe the physiological and morphological features of this model, and 3) to study PEEP in this model to evaluate indicators of "best PEEP".

Chapter VII

We developed a stable model of respiratory distress in pigs with oleic acid, that fulfilled the essential clinical criteria of the adult respiratory distress syndrome. Eight pigs (9.1 $\pm$ 0.7 kg) were anesthetized with pentobarbital and paralyzed with tubocurarine. The animals were mechanically ventilated with an F_{IO2} of 0.6, an I:E-ratio 2:3 and a P_{EEP} of 2 cmH_2O. A series of injections (0.1 ml) of oleic acid, dissolved 1:1 in 96 % alcohol, was given into the right atrium. The number of injections necessary to induce a stable model of respiratory distress (P_{aO2} = 54.9 $\pm$ 6.1 mmHg, sd, n=6) was different for different animals. We hypothesized that a threshold concentration had to be surpassed depending on the binding capacity of albumine for unsaturated fatty acids. Multiple small doses in stead of a bulk dose resulted in a low mortality. The distress model was stable for about 4 h. Such a stable period allows a diversity of studies on early respiratory distress.

Chapter VIII

Distribution and severity of active vasoconstriction of muscular pulmonary arteries were morphometrically assessed in anesthetized, paralyzed and mechanically ventilated pigs with respiratory distress, induced by oleic acid. Vasoconstriction was deduced from the medial thickness which was measured and expressed as a percentage of external diameter. Six pigs received oleic acid (0.12 $\pm$ 0.07 $ml.kg^{-1}$), dissolved 1:1 in 96 % alcohol, in multiple injections of 0.1 ml.

Six pigs were used as controls. After the oleic acid injections a stable hypoxemia (P_{aO2} = 57 ± 8 mmHg, at F_{IO2} = 0.6) and pulmonary hypertension (mean P_{pa} = 36 ± 2 mmHg) were obtained for several hours. Electron microscopy revealed swelling of endothelial cells with signs of degeneration. Medial thickness was far greater in the oleic acid group than in the control group, overall mean values were 8.1 ± 3.2 % and 3.8 ± 1.7 % respectively ($p<0.001$). Arteries with prominent vasoconstriction were lying in clusters. This pattern was the same in dependent and non-dependent regions. We concluded that pulmonary hypertension in oleic acid induced respiratory distress, to a very large degree is caused by active vasoconstriction. Besides vasoconstriction, endothelial swelling and intravascular clotting may contribute to the development of pulmonary hypertension.

Chapter IX

This study was aimed at the evaluation of variables, which could serve as indicators of maximal oxygen transport in pigs with early respiratory distress after oleic acid administration in order to apply the "best" level of positive end-expiratory pressure, "best PEEP". Respiratory distress was induced in seven young pigs of 9.5 ± 0.5 kg (sd) body weight as described in Chapter VII. After the induction of respiratory distress the gas exchange and hemodynamic data were similar to those of the group of Chapter VII.

One hour after oleic acid administration, PEEP was increased in steps of 2 cmH_2O from $PEEP_2$ (PEEP of 2 cmH_2O) up to $PEEP_{12}$ and reset to $PEEP_2$. Each level was maintained for 30 min. P_{aO2} increased from 56 ± 7 mmHg at $PEEP_2$ to 226 ± 45 mmHg at $PEEP_{12}$. End-expiratory lung volume increased from 16 ± 3 $ml.kg^{-1}$ to 50 ± 3 $ml.kg^{-1}$. Central venous pressure increased and cardiac output decreased linearly. "Best PEEP" was individually different and on average at $PEEP_6$. Mixed venous P_{O2} ($P_{\bar{v}O2}$) and S_{O2} ($S_{\bar{v}O2}$) correlated significantly with oxygen delivery (D_{O2}) in four and three animals respectively. Physiological dead space, arterial to end tidal P_{CO2} gradient, total respiratory compliance and venous admixture did not correlate with D_{O2}. We concluded that $P_{\bar{v}O2}$ and $S_{\bar{v}O2}$ might be useful indicators of D_{O2} if oxygen uptake is constant.

Chapter X

A comparison was made between the lavage and oleic acid model. Respiratory distress was induced in the lavage model by a standardized regime of

lavages, whereas in the oleic acid treated group the regime had to be individually adapted to obtain a comparable hypoxemia in all animals. Most gas exchange and pulmonary variables were not significantly different between both models and a similar degree of lung injury according to criteria of the lung injury score, introduced by Murray et al. was observed. Both models showed an acute inflammation of the lungs with thickening of septa indicating edema. The involvement of granulocytes in the pathogenesis of the lung injury in both models is discussed. Total lung lavages induced respiratory distress mainly by a depletion of surfactant, whereas oleic acid injection mainly resulted in vascular lesions. Striking differences were observed in hemodynamic variables between the models. In the lavage model there was no indication of any depressive effect on heart and circulation, cardiac output and central venous pressure were unchanged. In the oleic acid model cardiac output was decreased and central venous pressure increased. In both models pulmonary arterial pressure (P_{pa}) was increased by active vasoconstriction. In the oleic acid group P_{pa} was higher than in the lavage animals despite a same degree of active vasoconstriction, indicating that additional factors contributed to the pulmonary hypertension. These factors were endothelial swelling, intravascular clotting and compression by edema. The formation of edema was more pronounced after oleic acid than in the lavage group. Application of PEEP resulted in the similar effects with regard to gas exchange, lung mechanics and circulation. In both models total respiratory compliance, arterial minus end-tidal carbon dioxide gradient, physiological dead space and venous admixture appeared not to be reliable indicators of maximum oxygen delivery, "best PEEP". Mixed venous oxygen tension and saturation, however, appeared to be reliable indicators of "best PEEP" in both models.

Finally, some directions for future research are discussed.

CHAPTER XII

SAMENVATTING

Hoofdstuk I

Respiratoire distress wordt gekenmerkt door een plotselinge kortademigheid, een daling van de arteriële zuurstofspanning en een toename van de stugheid van de long, zonder dat er een chronische longziekte of falen van het hart aan ten grondslag ligt. Respiratoire distress komt voor bij volwassenen als "adult respiratory distress syndrome" (ARDS) en bij pasgeboren kinderen als "infant respiratory distress syndrome" (IRDS). ARDS kan bij een veelheid van ziektetoestanden voorkomen.

Een van de voornaamste verschijnselen is destructie van de alveolo-capillaire membraan van de long, i.c. de lucht-bloed barriere, waardoor vocht uit de bloedbaan in de longblaasjes komt en de opname van zuurstof en de afgifte van koolzuur bemoeilijkt wordt. IRDS komt voornamelijk voor bij te vroeg geboren babies en wordt veroorzaakt door een tekort aan surfactant, oppervlakte spanningsverlagende stoffen, in de long met als gevolg het dichtvallen van longblaasjes, atelectase. Iedere ARDS en IRDS patient is verschillend. Dit maakt het doen van betrouwbare uitspraken over bijvoorbeeld therapie moeilijk. In dieren kan respiratoire distress op een gestandaardiseerde manier opgewekt worden, waardoor bij ieder dier een zelfde mate van respiratoire distress ontstaat. Bij veel diermodellen die in de literatuur zijn beschreven, is de long nauwelijks aangedaan of is er geen stabiele periode van respiratoire distress. In dit proefschrift worden twee diermodellen in biggen beschreven. Het eerste model wordt opgewekt door het uitwassen van surfactant, longlavage, als analogon van surfactant tekort. Het tweede model wordt verkregen door het intraveneus inspuiten van een vetzuur, oliezuur, als analogon voor ARDS dat onstaat na vetembolie. Meer algemene informatie over ieder van deze modellen en een literatuur overzicht staan in hoofdstuk II en hoofdstuk VI van dit proefschrift.

Mechanische beademing waarbij een positieve druk aan het einde van de uitademing in de longen blijft bestaan, "positive end-expiratory pressure" (PEEP), wordt meestal als therapie gebruikt bij zowel ARDS als IRDS. PEEP heeft echter negatieve effecten op de circulatie, zodat deze therapie niet onbegrensd gegeven kan worden. In de literatuur is er een verschil van mening over de vraag aan de hand van welke variabelen PEEP ingesteld dient te worden teneinde een maximaal

zuurstofaanbod aan de weefsels, "best PEEP", te verkrijgen. In dit proefschrift worden verschillende indicatoren van "best PEEP" op hun waarde getoetst in beide diermodellen van respiratoire distress.

Hoofdstuk II

Surfactant zorgt voor een verlaging van de oppervlaktespanning in de long. Wanneer er een tekort aan surfactant bestaat, treedt atelectase op. Een tekort aan surfactant, surfactant deficiëntie, is belangrijk bij verdrinking en IRDS, maar is ook als bijkomende factor van belang voor de pathogenese, het ziektemakende proces van ARDS. Ter bestudering van effecten van surfactant deficiëntie kunnen totale longlavages gebruikt worden. Hierbij wordt surfactant uit de long gewassen. In de literatuur over het lavage model in dieren werden de beademingsomstandigheden vaak gewijzigd tijdens de proeven. Dit maakt onderlinge vergelijking en de bestudering van effecten van therapie moeilijk. Ook waren gegevens over de stabiliteit van het lavage model schaars.

Effecten van PEEP op het zuurstofaanbod aan de weefsels zijn onder omstandigheden van surfactant deficiëntie nauwelijks bestudeerd, laat staan dat indicatoren voor een optimale instelling hiervan werden geëvalueerd.

Hoofdstuk III

Het ontwikkelen van een lavage model werd in verschillende systematische stappen uitgevoerd. In een eerste serie van experimenten werden de effecten van een enkele longlavage nagegaan. Een lavage werd uitgevoerd door de beademing te stoppen en via de luchtpijp 35 ml fysiologische keukenzout oplossing (circa 0.9 %) per kg lichaamsgewicht in de longen te laten lopen, deze er uit te hevelen, nogmaals er in te laten lopen en vervolgens er weer uit te hevelen. Een dergelijke lavage duurde 90 seconden. De zuurstofspanning bleek te dalen na zo'n lavage, maar na verloop van tijd trad herstel op (Figuur 3.1.a). Een tweede lavage volgde zodra de zuurstofspanning niet verder meer herstelde. Weer daalde de zuurstofspanning, maar nu sterker. Ook deze werd gevolgd door een herstel, zij het partiëel. Na nog een lavage bleek er een stabiel lage zuurstofspanning te ontstaan.

In een tweede serie van experimenten werd na een dubbele lavage, d.w.z. twee lavages met een tussentijd van 5 minuten, een sterke daling van de zuurstofspanning waargenomen, die enige uren later meer of minder hersteld was (Figuur 3.1.b). Wanneer een tweede paar lavages gedaan werd op het moment dat geen

verder herstel meer optrad, werd de zuurstofspanning sterk verlaagd en bleef op dit niveau gedurende verschillende uren. In een volgende serie van experimenten werd niet het herstel van de zuurstofspanning afgewacht en werd het tweede paar lavages een uur na het eerste paar uitgevoerd (Figuur 3.1.c). Ook dit tweede paar had tot gevolg dat de zuurstofspanning sterk verlaagd werd en op dit lage niveau stabiel bleef, en wel gedurende circa 6 uur. Werd echter het tweede paar lavages een half uur na het eerste paar uitgevoerd, dan bleek er nog wel herstel op te treden (Figuur 3.1.d). Geconcludeerd werd dan ook dat het tweede paar lavages tussen een half en één uur na de eerste lavages moet worden uitgevoerd. Er werden verder geen dieren opgeofferd om deze tijd precies vast te stellen, aangezien wij een spreiding in deze periode verwachtten, en een triviale tijdswinst van b.v. 15 minuten voor de ontwikkeling van het model weinig betekenis had. Onze hypothese was dat door de uitwas van surfactant tijdens het eerste paar lavages de cellen die het surfactant produceren, pneumocyten II, worden gestimuleerd tot het uitstorten van surfactant. Vervolgens wordt dit surfactant door het tweede paar lavages een uur later ook uitgewassen met als gevolg dat geen herstel meer kan optreden. Wanneer echter het proces van uitstorten van surfactant uit de pneumocyten II nog niet voltooid is op het moment van het tweede lavage paar kan nog wel enig herstel van de longontplooiing en daarmee van de zuurstofspanning optreden. Deze veronderstelling werd gesteund door onderzoek van de pneumocyten II. Hierin bleken aan het einde van de experimenten de structuren die het surfactant bevatten, de lamellar bodies, leeg te zijn (Figuur 3.7). Onze conclusie was dan ook dat het uitvoeren van twee paar lavages met een uur tussentijd, een stabiele respiratoire distress tot gevolg heeft. Deze respiratoire distress voldeed aan de criteria zoals deze in de kliniek gebruikt worden. Bij dit model bleven het hartminuutvolume, de arteriële en de centraal veneuze bloeddruk op hetzelfde niveau als voor de lavages. Het zuurstofaanbod aan de weefsels was verminderd en de bloeddruk in de longslagader aanmerkelijk toegenomen. In de periode na de lavages bleven deze variabelen op een stabiel niveau.

Hoofdstuk IV

Patienten met respiratoire distress hebben gewoonlijk een verhoging van de bloeddruk in de longslagaderen, pulmonale hypertensie. In de serie experimenten met respiratoire distress door longlavages in biggen trad ook een sterke pulmonale hypertensie op. Nagegaan werd of deze pulmonale hypertensie berustte op actieve vasoconstrictie, d.w.z. vernauwing van de longvaatjes door samentrekking van het gladde spierweefsel in de wand, en hoe deze vasoconstrictie verdeeld is over

de long. Actieve vasoconstrictie werd vastgesteld door meting van de dikte van de wand, i.c. de media, als percentage van de totale diameter van de musculaire pulmonaal arteriën. Per dier werden vier longkwabben onderzocht en per kwab 25 musculaire arteriën. Na 6 uren van respiratoire distress werden de longen onmiddelijk na de dood gefixeerd met formaline via de luchtpijp, trachea. Op een later tijdstip werden uitsnijdingen verricht en coupe's gemaakt. Als controle diende een groep dieren, die gedurende een zelfde periode mechanisch beademd werd en waarbij de twee paar lavages alleen in schijn werden uitgevoerd door de beademing te stoppen op dezelfde momenten en gedurende dezelfde tijd als in de serie met echte lavages. Bij deze schijnlavages werd dus geen fysiologische zoutoplossing in en uit de longen geheveld. De media-dikte bleek in alle longkwabben sterk toegenomen te zijn in de dieren na lavages (Figuur 4.1). Dit wijst erop dat de pulmonale hypertensie bij lavage geïnduceerde respiratoire distress voornamelijk berust op actieve vasoconstrictie. Voorts bleek de verdeling binnen de longkwabben onregelmatiger verdeeld te zijn dan in de controles (Figuur 4.3). Dit gold voor alle bestudeerde longkwabben. Actieve vasoconstrictie na longlavages wordt waarschijnlijk voornamelijk veroorzaakt doordat locaal in de longen een lage zuurstofspanning heerst, hypoxie. Mediatoren, stoffen afkomstig van de na lavages sterk toegenomen hoeveelheid witte bloedcellen in en bij de longblaasjes, dragen waarschijnlijk ook bij tot het ontstaan van de vasoconstrictie.

Hoofdstuk V

Het belangrijkste doel van positieve eind-expiratoire druk (PEEP) is het verbeteren van het zuurstofaanbod naar de weefsels. Het zuurstofaanbod wordt vooral bepaald door het zuurstofgehalte van het bloed en de hoeveelheid bloed die het hart per minuut uitpompt, het hartminuutvolume. De effecten van een stapsgewijze verhoging van PEEP met telkens 2 cmH_2O druk werden bestudeerd na lavages. Na iedere stap werd de PEEP gedurende een half uur constant gehouden. De PEEP werd tot een niveau van 14 cmH_2O verhoogd. Hierna werd teruggegaan naar een PEEP van 2 cmH_2O. PEEP had tot ongeveer 6 cmH_2O een sterke stijging van het zuurstofgehalte tot gevolg en gelijktijdig een daling van het hartminuutvolume (Figuur 5.1 en 5.4.a). Boven 6 cmH_2O steeg het zuurstofgehalte niet verder, maar daalde wel het hartminuutvolume bij verhoging van de PEEP. Dit had tot gevolg dat er een maximum van het zuurstofaanbod, "best PEEP", was rond 6 cmH_2O (Figuur 5.5).

Meting van het hartminuutvolume is essentieël voor de bepaling van het zuurstofaanbod. Deze meting is echter tamelijk ingrijpend voor de patient. Het is daarom van belang om bij dieren met respiratoire distress te onderzoeken of er

andere variabelen zijn die een indicatie geven van het zuurstofaanbod. In het onderzoek met PEEP hebben wij dit voor een aantal bepalingen nagegaan. Dit onderzoek viel negatief uit voor de in de literatuur gesuggereerde bepalingen, zoals stugheid van de long of het omgekeerde daarvan de longcompliantie, het verschil tussen arteriële kool-zuurspanning en die aan het einde van de uitademing, dode ruimte van de beademingslucht en de veneuze bijmenging (Tabel 5.2 en 5.3). Na lavages bleken de gemengd veneuze zuurstofspanning en saturatie een goede voorspellende waarde te hebben voor het maximale zuurstofaanbod (Tabel 5.2 en 5.3, Figuur 5.1.c, 5.1.d en 5.5). Geconcludeerd werd dat deze beide variabelen tezamen met het zuurstofaanbod de meest betrouwbare bepalingen zijn bij de behandeling van respiratoire distress met PEEP.

Hoofdstuk VI

De diagnose vetembolie werd voor het eerst gesteld in 1873. Enkele klinische aspecten worden in dit hoofdstuk beschreven. Voor de pathogenese van het vetembolie syndroom zijn verschillende factoren van belang. Besproken worden de oorzaken van vetembolie, de herkomst van het vet, de bijdrage van bepaalde stoffen in de long, zoals lipase dat neutraal vet splitst in vrije vetzuren als oliezuur en linolzuur, en de bijdrage van het complement- en stollingssysteem. Veel dierexperimenteel onderzoek is gedaan sinds de ontdekking van dit syndroom. Hierbij bleek o.a. dat oliezuur veel meer schade aanbrengt dan neutraal vet. Reeds in 1968 werd injectie in de bloedvaten van oliezuur beschreven voor het nabootsen van een beeld van respiratoire distress in dieren dat veel gelijkenissen vertoont met het klinische beeld van vetembolie.

In dieren werd aangetoond dat oliezuur een direct schadelijke werking uitoefent op de bekleding, endotheel, van de longcapillairen. Ophoping van bloedplaatjes en witte bloedcellen in de long na de toediening van oliezuur draagt vermoedelijk bij tot de longafwijkingen. In de meeste publicaties staat dat oliezuur werd toegediend als een grote "bolus" injectie of als een continu infuus. Op deze wijze werd slechts een geringe mate van respiratoire distress verkregen die ook nog veel verschillen vertoonde. De sterfte van de dieren daarbij was ongeveer 30 %. Het doel van ons onderzoek was om een sterke mate van respiratoire distress op te wekken met oliezuur in biggen. Gestreefd werd om te voldoen aan de voor patienten geldende criteria van respiratoire distress. Tevens werd gestreefd naar een lage sterfte en een stabiliteit van het model gedurende enige uren, zodat effecten van behandeling bestudeerd kunnen worden.

Om een respiratoire distress in biggen te verkrijgen werd een reeks van injecties van 0.1 ml oliezuur één op één vermengd met 96% alcohol in het rechter atrium gespoten na opvulling van de dieren met een bloedplasma vervangende vloeistof, dextran, in een hoeveelheid van 10 $ml.kg^{-1}$. Deze injecties werden met tussenpozen van minstens 90 seconden op geleide van de arteriële zuurstofspanning toegediend, in die zin dat gestopt werd, indien de arteriële zuurstofspanning tussen de 50 en 60 mmHg was. Hierbij werd beademd met een zuurstof-luchtmengsel waarvan de zuurstoffractie 0.6 was. Met deze procedure werd voldaan aan één van van de belangrijkste klinische criteria van respiratoire distress. Na bijna elke oliezuurinjectie werd binnen enkele seconden een stijging in de druk van de longslagader gezien gevolgd door een stijging van de centraal veneuze druk en een daling van de bloeddruk (Figuur 7.1). Werden na een oliezuurinjectie te heftige reacties waargenomen in de bovengenoemde bloeddrukken, dan werd gewacht met verdere toediening totdat deze weer stabiel waren (dus langer dan 90 seconden).

Het bleek dat het aantal benodigde injecties om een stabiele respiratoire distress in alle dieren te verkrijgen (Figuur 7.2), aanzienlijk verschilde tussen de verschillende dieren (Tabel 7.2). Mede op grond van literatuurgegevens werd geconcludeerd dat een drempelconcentratie van oliezuur overschreden moet worden om een effect te krijgen. Deze drempelconcentratie zou afhankelijk zijn van de bindingscapaciteit van albumine voor ongesatureerde vetzuren. Zolang vetzuren aan albumine gebonden worden, zijn deze nog niet schadelijk. Doordat de bindingscapaciteit van albumine voor vetzuur per dier kan verschillen zijn er verschillende hoeveelheden vetzuur nodig om de drempelwaarde te overschrijden. Dit is waarschijnlijk de belangrijkste reden dat onze wijze van injecteren in meerdere kleine doses in plaats van oliezuurtoediening in één grotere hoeveelheid, zoals meestal werd toegepast, een lagere sterfte tot gevolg had.

Na de oliezuur injecties bleef de zuurstofspanning op een stabiel niveau gedurende minimaal 4 uur (Figuur 7.3.a). Het hartminuutvolume en daardoor ook het zuurstofaanbod aan de weefsels waren sterk verlaagd, maar stabiel (Figuren 7.8.a en 7.8.e). De druk in de longslagader was sterk verhoogd en eveneens stabiel (Figuur 7.8.c). In een dergelijke stabiele periode kan betrouwbaar onderzoek naar ontstaansmechanismen (pathogenese) en therapie van respiratoire distress gedaan worden.

Het pathologisch anatomisch beeld van het longweefsel werd gekenmerkt door locale ophopingen van bloed ofwel stuwingsverschijnselen, vochtophoping in de wanden van de longblaasjes ofwel interstitiëel oedeem, beschadiging van de wanden van de longblaasjes en de longcapillairen, stolselvorming in de

bloedvaatjes en een uitgebreide ophoping van granulocyten, een bepaald type witte bloedcellen, in en bij de longblaasjes (Figuren 7.9 tm 7.11).

Hoofdstuk VIII

Intraveneuze oliezuurinjectie heeft een sterke stijging van de druk in de longslagader, pulmonale hypertensie, tot gevolg. In de literatuur werd gesuggereerd dat vasoconstrictie, ofwel vernauwing d.m.v. samentrekking van het spierweefsel in de wand, van arteriën in de long een belangrijke factor zou kunnen zijn die deze drukstijging veroorzaakt. Om meer inzicht te krijgen in de ernst en verdeling van vasoconstrictie van de musculaire pulmonaal arteriën in het oliezuur model werd een morfometrische studie verricht. De meting van de media-dikte als maat voor actieve vasoconstrictie werd overeenkomstig die na lavage (Hoofdstuk IV) uitgevoerd.

Na oliezuur injectie bleek er een ernstige mate van actieve vasoconstrictie van de musculaire pulmonaal arteriën te bestaan (Figuren 8.2, 8.3.a en tabel 8.2). Arteriën met vasoconstrictie leken in clusters te liggen. Dit patroon was hetzelfde in alle longdelen, een hydrostatisch effect kon worden uitgesloten. Naast de vasoconstrictie werd zwelling van endotheel met tekenen van degeneratie (Figuur 8.3.b) en stolselvorming (Figuur 8.1) in bloedvaten gezien. Geconcludeerd werd dat de pulmonale hypertensie na oliezuur vooral veroorzaakt wordt door vasoconstrictie. Factoren die bijdragen aan de pulmonale hypertensie in het oliezuurmodel zijn naar alle waarschijnlijkheid endotheelzwelling, vorming van stolsels in de bloedvaten en dichtdrukken van de vaten door longoedeem.

Hoofdstuk IX

De effecten van positieve eind-expiratoire druk (PEEP) werden bestudeerd in het oliezuur model. Net als na lavages werd PEEP in stappen van 2 cmH_2O verhoogd en werd ieder niveau gedurende een half uur bestudeerd. Onder invloed van PEEP verbeterde de gaswisseling (Figuur 9.2). Het hartminuutvolume, echter, daalde (Figuur 9.7.a). In de individuele dieren werd steeds een maximum van het zuurstofaanbod gevonden, dat tussen 4 en 8 cmH_2O lag. De indicatoren, die volgens de literatuur dit PEEP niveau met maximaal zuurstofaanbod, "best PEEP", voorspelden, zoals totale respiratoire compliantie, dode ruimte van het beademingsvolume en het verschil tussen de arteriële koolzuurspanning en die aan het einde van de uitademing, deden dat in het oliezuur model niet (Tabel 9.2 en 9.3).

Variabelen, die volgens het eigen onderzoek geschikte indicatoren van "best PEEP" zouden zijn waren de gemengd veneuze zuurstofspanning en in mindere mate de gemengd veneuze zuurstofsaturatie (Figuren 9.2.c en 9.2.d). Deze variabelen vertoonden een significante correlatie met het zuurstofaanbod (Tabel 9.3).

Hoofdstuk X

In dit hoofstuk wordt een vergelijking tussen het lavage model en het oliezuur model gemaakt. Op grond van de pathogenese van de beide modellen was de wijze van opwekken sterk verschillend: lavages werden volgens een standaard regime gegeven, terwijl oliezuur individueel gedoseerd werd. Lavages werkten via het weg nemen van surfactant (Figuur 3.7) en oliezuur via schade aan het endotheel (Figuur 7.11). Ondanks de verschillende aangrijpingspunten was het in beide modellen mogelijk om een gelijkwaardige respiratoire distress op te wekken die voldeed aan klinische criteria (Tabel 10.1). Volgens een in de literatuur beschreven score van longschade (Tabel 1.1) was de schade in de longen gelijk in beide modellen. Zowel na lavage als na oliezuur toediening was er een acute ontstekingsreactie in de longen aanwezig. Vasoconstrictie en verhoging van de druk in de longslagader, pulmonale hypertensie, werden in beide modellen gevonden. In de oliezuur groep was de druk in de longslagader echter hoger dan in de lavage groep bij eenzelfde mate van vasoconstrictie volgens het morfometrisch onderzoek (Tabellen 4.2 en 8.2). Dit betekent dat er in het oliezuurmodel additionele factoren aan de pulmonale hypertensie bijdroegen. Deze factoren waren waarschijnlijk zwelling van endotheel en aanwezigheid van fibrine-thrombi in de vaten (Figuren 8.1 en 8.3.b). Naast een hoge druk in de longslagader was het hartminuutvolume verlaagd en de centraal veneuze druk verhoogd in het oliezuur model (Tabel 10.1), wat mogelijk kan betekenen dat de hartfunctie verminderd was. In het lavage model werden zulke effecten op de circulatie niet gezien.

De effecten van PEEP waren in beide modellen gelijkwaardig (Figuren 10.1 tm 10.3). Wat betreft indicatoren voor "best PEEP" was het resultaat ook hetzelfde: longcompliantie, het verschil tussen de arteriële koolzuurspanning en die aan het eind van de uitademing, dode ruimte van het beademingsvolume en veneuze bijmenging bleken geen betrouwbare voorspellers te zijn voor het maximale zuurstofaanbod aan de weefsels. In beide modellen waren de gemengd veneuze zuurstofspanning en -saturatie de beste indicatoren van het maximale zuurstofaanbod.

Beademing heeft als gunstig effect dat de gaswisseling in de longen, en

daarmee het zuurstofgehalte van het bloed, gunstig wordt beinvloed door ontplooiing van dichtgeklapte longblaasjes. Een negatief effect op de gasoverdracht is vermindering van het longbloedvolume, zoals werd gevonden in biggen met gezonde longen. Vragen die nader onderzoek vereisen, zijn: 1) in welke mate overweegt het gunstige effect over het ongunstige in biggen met zieke longen, 2) in hoeverre is dit verschillend bij de beide "respiratoire distress" modellen, en 3) in welke mate kan het gunstige effect positief en het ongunstige effect negatief beinvloed worden door verandering van beademingscondities.

List of abbreviations:

ARDS	adult respiratory distress syndrome
C_{aO2}	arterial oxygen content
C_{rs}	total respiratory system compliance
C_{dyn}	dynamic lung compliance
D_{O2}	oxygen delivery
ECG	electrocardiogram
F_{EO2}	expiratory oxygen fraction
F_{IO2}	inspiratory oxygen fraction
h	hour
Hb	hemoglobin
HCO_3^-	standard bicarbonate
IRDS	Infant Respiratory Distress Syndrome
PEEP	positive end-expiratory pressure
$PEEP_2$	positive end-expiratory pressure at 2 cmH_2O
P_{ao}	aortic pressure averaged over a ventilatory cycle
P_{cv}	central venous pressure averaged over a ventilatory cycle
$P_{cv,tm}$	transmural central venous pressure averaged over a ventilatory cycle
P_{pa}	pulmonary arterial pressure averaged over a ventilatory cycle
$P_{pa,tm}$	transmural pulmonary arterial pressure averaged over a ventilatory cycle
P_{it}	intrathoracic pressure
P_{AO2}	alveolar oxygen tension
P_{aCO2}	arterial carbon dioxide tension
P_{aO2}	arterial oxygen tension
$P_{\bar{v}O2}$	mixed venous oxygen tension
$P_{\bar{E}CO2}$	mixed expiratory carbon dioxide tension
$P_{T,p}$	peak tracheal pressure
Q'_s/Q'_t	venous admixture
Q'_t	cardiac output
R	respiratory quotient
RDS	respiratory distress syndrome
t	time
s	second
S_{aO2}	arterial oxygen saturation
$S_{\bar{v}O2}$	mixed venous oxygen saturation
sd	standard deviation
V_D/V_T	physiological dead space
V_{EE}	end-expiratory lung volume
V_{EEin}	V_{EE} determined with an open wash-in method for helium
V_{EEout}	V_{EE} determined with an open wash-out method for helium
V'_{O2}	oxygen uptake
ZEEP	zero end-expiratory pressure

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Curriculum Vitae
van
Roos M.J.L. van der Heijde

Roos van der Heijde werd geboren op 26 december 1961 te Haarlem. In 1979 behaalde zij het diploma Gymnasium 6 aan het 's-Gravenhaags Christelijk Gymnasium "Sorghvliet". In datzelfde jaar werd de studie Geneeskunde aangevangen aan de Rijksuniversiteit te Leiden. In 1987 werd het arts-examen behaald. Gedurende de studie verrichtte zij onderzoek naar de waterstof-ademtest bij lactose-intolerantie op de afdeling Kindergeneeskunde (hoofd: Prof.Dr. L.J. Dooren) van het Academisch Ziekenhuis te Leiden. In 1983-1984 was zij in hetzelfde ziekenhuis werkzaam als student-assistent op de afdeling Longziekten (hoofd: Prof.Dr. J.H. Dijkman), alwaar onderzoek werd gedaan naar de 10-jaarsoverleving van bronchuscarcinoom. Van 1 januari 1988 tot voorjaar 1992 werkte zij bij het Pathofysiologisch Laboratorium van de afdeling Longziekten aan de Erasmus Universiteit te Rotterdam als Assistent in Opleiding (A.I.O.) onder leiding van Prof.Dr. A. Versprille aan dit promotie-onderzoek. In het kader van de A.I.O.-opleiding werd onderwijs gevolgd in Engels, Biostatistiek, Klinische Farmacologie, Drug Research (Janssen Pharmaceutica), Wetgeving/Ethiek, Epidemiologie en Besliskunde, Systeemtheorie, Respiratoire Intensive Care en Longfunctie. Vanaf 1 april 1992 is zij in opleiding tot longarts in het Academisch Ziekenhuis Dijkzigt te Rotterdam (opleider: Prof.Dr. C. Hilvering). In het kader daarvan werkt zij thans als arts-assistent op de afdeling Interne Geneeskunde II (hoofd: Prof. J.H.P. Wilson) van het Academisch Ziekenhuis Dijkzigt te Rotterdam.

Curriculum Vitae
van
Hans P. Grotjohan

Hans Grotjohan werd op 19 december 1958 geboren te Apeldoorn. In 1977 behaalde hij het diploma Atheneum B aan het Myrtus College te Apeldoorn. Na 1 jaar Farmacie studie (i.v.m. uitloting voor Geneeskunde) begon hij in 1978 met de studie Geneeskunde aan de Rijksuniversiteit te Utrecht. In 1985 werd het artsexamen behaald. In 1981 deed hij tijdens een wetenschappelijke stage onderzoek naar het effect van irradiatie op het plasma groeihormoon gehalte bij acromegalie patiënten op de afdeling Endocrinologie van het Academisch Ziekenhuis Utrecht (Prof.Dr. F. Schwartz, Dr. R.J.M. Croughs). Daarnaast was hij in 1981 en 1982 werkzaam als student-assistent bij het eerste- en tweedejaars snijzaalpraktikum op het Laboratorium voor Medische Anatomie en Embryologie te Utrecht (Prof.Dr. W.J. van Doorenmaalen). In 1982 was hij als docent verbonden aan de opleiding ziekenverzorging bij het Psycho-Geriatrisch verpleeghuis Het Immendaal te Beekbergen, alwaar hij de lessen pathologie verzorgde. Van 1985-1986 werkte hij als arts-assistent op de afdeling Interne Geneeskunde van de Stichting Ziekenhuiscentrum Apeldoorn, lokatie Lukas Ziekenhuis (Dr. G. Fedder). In 1987 werd de militaire dienstplicht vervuld als Eerste Luitenant der Koninklijke Landmacht (Rijschool Venlo). Op 1 januari 1988 trad hij als Assistent in Opleiding (A.I.O.) in dienst van de Erasmus Universiteit Rotterdam. Tot 1 januari 1992 werkte hij op het Pathofysiologisch Laboratorium van de afdeling Longziekten aan dit promotie-onderzoek onder leiding van Prof.Dr. A. Versprille. In het kader van de A.I.O.-opleiding werd onderwijs gevolgd in Engels, Biostatistiek, Klinische Farmacologie, Epidemiologie en Besliskunde, Drug Research (Janssen Pharmaceutica), Wetgeving/Ethiek, Systeemtheorie, Respiratoire Intensive Care en Longfunctie. Thans is hij werkzaam als arts op de Longfunctie afdeling van het Academisch Ziekenhuis Dijkzigt te Rotterdam.